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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03290490.6

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Novel compounds and methods of treating cell profilerative diseases, retinopathies and arthritis

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Novel compounds and methods of treating cell proliferative diseases, retinopathies and arthritis

The invention relates to compounds and their uses, particularly in the pharmaceutical industry. The invention discloses compounds having anti-proliferative and antiangiogenic activities, as well as methods for treating various diseases associated with abnormal cell proliferation, including cancer, or associated with unregulated angiogenesis including growth and metastasis of solid tumors, ocular diseases and especially retinopathies, or arthritis, by administering said compounds. It further deals with pharmaceutical compositions comprising said compounds, more particularly useful to treat cancers, ocular diseases and arthritis.

Cancer is still one of the leading causes of death in developed countries, as cancer affects all ages, sexes, racial and ethnic groups. According to the American Association for Cancer Research, one out of five deaths in the US is caused by cancer. Worldwide, the most predominant cancer sites are lung (14%), prostate (13%), breast (11%) and colorectal (11%) (data obtained from the Cancer Statistic Branch, NCI).

Cancer rate is increasing in developed countries in spite of falling incidence of several cancers such as prostate cancer (due to detection programs) or lung cancer in men (due to prevention programs). Among the fastest increasing cancer rates are non-Hodgkin 's lymphoma cancer and melanoma (3% annual rise) in the US (The Annual Report to the Nation on the Status of Cancer, 1973-1997).

Unlike cancer incidence, cancer deaths have declined in developed countries. This is due in part to better therapy designs but also to prevention programs and better detection of some cancers at an earlier stage.

However, in spite of higher achievements in treatment and prevention of cancers, several improvements are awaited for:

- effective therapies for early stage cancer to reduce relapses,

- alternative therapies for curing tumors refractory to standards therapies,
- alternative therapies for curing metastatic cancers
- less toxic drugs, and
- better delivery systems.

Inhibitors of cell signaling pathways could represent such a new alternative therapy by addressing the first three issues, when used alone or in combination with standard chemotoxic drugs. However as cell signaling pathways are ubiquitous, the toxicity of these inhibitors could be compared to the toxicity of standard chemotoxic drugs. To reduce their toxicity and enhance their tissue specificity, these inhibitors could be coupled to appropriate drug delivery systems.

U.S. Patent No. 4,590,201 discloses compound L651582, a cell signaling inhibitor. This compound inhibits proliferation and inflammation by affecting the biochemical pathways necessary for signal processing in the cell. It is an indirect blocker of the effector enzymes which produce the second messengers necessary to induce growth.

Angiogenesis is a fundamental process by which new blood vessels are formed and is essential to a variety of normal body activities (such as reproduction, development and wound repair). Although the process is not completely understood, it is believed to involve a complex interplay of molecules which both stimulate and inhibit the growth of endothelial cells, the primary cells of the capillary blood vessels. Under normal conditions, these molecules appear to maintain the microvascularity in a quiescent state for prolonged periods which may last for as long as weeks or, in some cases, decades. When necessary however (such as during wound repair), these same cells can undergo rapid proliferation and turnover within a 5 day period.

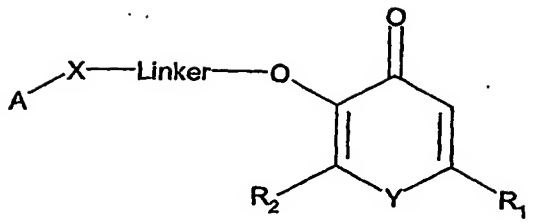
Although angiogenesis is a highly regulated process under normal conditions, many diseases are driven by persistent unregulated angiogenesis. Otherwise stated, unregulated angiogenesis may either cause a particular disease directly

or exacerbate an existing pathological condition. For example, ocular neovascularization has been implicated as the most common cause of blindness and dominates approximately 20 eye diseases. In certain existing conditions such as arthritis, newly formed capillary blood vessels invade the joints and destroy cartilage. In diabetes, new capillaries formed in the retina invade the vitreous, bleed, and cause blindness. Growth and metastasis of solid tumors are also angiogenesis-dependent. It has been shown for example that tumors which enlarge to greater than 2 mm, must obtain their own blood supply and do so by inducing the growth of new capillary blood vessels. Once these new blood vessels become embedded in the tumor, they provide a means for tumor cells to enter the circulation and metastasize to distant sites, such as liver, lung or bone.

Although several angiogenesis inhibitors are currently under development for use in treating angiogenesis diseases, there are disadvantages associated with several of these compounds. For example, suramin is a potent angiogenesis inhibitor, but causes severe systemic toxicity in humans. Other compounds, such as retinoids, interferons and antiestrogens are safe for human use but have only a weak anti-angiogenic effect. Still other compounds may be difficult or costly to make.

The present invention now relates to the identification and characterization of a new class of compounds which present an anti-cell proliferation effect, more particularly on tumor cells and also an antiangiogenic effect. Advantageously, these compounds will inhibit or reverse malignant cell phenotypes in a wide array of human tissues, have little or no effect on normal cell physiology, will be highly active so that a limited number of treatments will be needed for each patient, and will have excellent bio availability and pharmacokinetic properties.

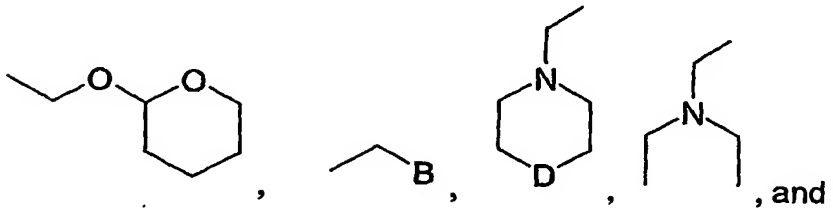
Accordingly, one aspect of the invention is to provide a compound having a general formula (I) :



(I)

wherein:

R_1 is selected from the group consisting of :



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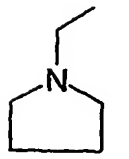

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R_2 represents a hydrogen atom, an alkyl or alkenyl group containing from 3 to 6 carbon atoms;

B represents an halogen atom, preferably chlorine, a hydroxyl group, a -O-CH_2-O-CH_3 (MOM) group, a -O-CH_2-O-CH_2-CH_2-O-CH_3 (MEM) group, a -OSO_2-alkyl group or a –$OSi(CH_3)_2tBu$;

D represents an oxygen atom, NR_3, CR'R" or a sulfur atom;

X represents an oxygen atom, a sulfur atom or a radical –NR_4-;

Y represents an oxygen atom, a sulfur atom or a radical $-NR_4-$;

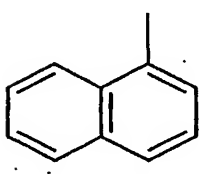
R_3 represents a hydrogen, an alkyl group or a SO_2-alkyl group;

R' and R", identical or different, represent an hydrogen atom or an alkyl radical;

R_4, identical or different, is selected from a group consisting of a hydrogen atom, an alkyl group having from 1 to 10 carbon atoms, an aryl and an aralkyl ;

"linker" represents $(CH_2)_n$, wherein n represents an integer between 1 and 10 inclusive or a aryldialkyl (preferably xylenyl) group;

A represents a group selected from :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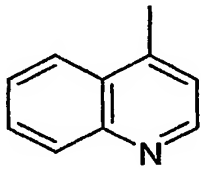
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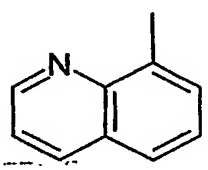
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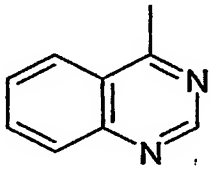
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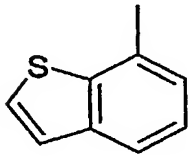

,

optionnally A is substituted,

its tautomers, optical and geometrical isomers, racemates, salts, hydrates and mixtures thereof.

The compounds of the present invention may have one or more asymmetric centers and it is intended that stereoisomers (optical isomers), as separated, pure or partially purified stereoisomers or racemic mixtures thereof are included in the scope of the invention.

The present invention also relates to pharmaceutical compositions comprising at least one compound as defined above in a pharmaceutically acceptable support, optionally in association with another active agent.

The pharmaceutical composition is more particularly intended to treat diseases of diseases associated with abnormal cell proliferation, such as cancers, or diseases associated with unregulated angiogenesis including growth and metastasis of solid tumors, ocular diseases and especially retinopathies (including diabetic retinopathies, retinal degenerative diseases, Age-Related Macular Degeneration (ARMD)), or arthritis.

The present invention also relates to the use of a compound as defined above, for the manufacture of a medicament for the treatment of diseases associated with abnormal cell proliferation, such as cancers or associated with unregulated angiogenesis, including growth and metastasis of solid tumors, ocular diseases and especially retinopathies (including diabetic retinopathies, retinal degenerative diseases, Age-Related Macular Degeneration (ARMD)), or arthritis.

The present invention also includes methods of treating diseases associated with unregulated angiogenesis including growth and metastasis of solid tumors, ocular diseases and especially retinopathies and arthritis and diseases associated with abnormal cell proliferation, such as cancers, comprising the administration to a subject in need thereof of an effective amount of a compound as defined above.

As will be further disclosed in this application, the compounds according to this invention have strong cell proliferation inhibitory activity and are effective at reducing or arresting growth of proliferating cells such as tumor cells.

Preferred embodiments

Within the context of the present application, the terms alkyl and alkoxy denote linear or branched saturated groups containing from 1 to 10 carbon atoms. An alkoxy group denotes an -O-alkyl group.

The alkyl groups may be linear or branched. Examples of alkyl groups having from 1 to 10 carbon atoms inclusive are methyl, ethyl, propyl, isopropyl, t-butyl, n-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, 2-ethylhexyl, 2-methylbutyl, 2-methylpentyl, 1-methylhexyl, 3-methylheptyl and the other isomeric forms thereof. Preferably, the alkyl groups have from 1 to 6 carbon atoms.

The alkenyl groups may be linear or branched. Examples of alkenyl containing from 3 to 6 carbon atoms are 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl and the isomeric forms thereof.

The term aryl includes any aromatic group comprising preferably from 5 to 14 carbon atoms, preferably from 6 to 14 carbon atoms, optionally interrupted by one or several heteroatoms selected from N, O, S or P. Most preferred aryl groups are mono- or bi-cyclic and comprises from 6 to 14 carbon atoms, such as phenyl, α-naphtyl, β-naphtyl, antracenyl, or fluorenyl group.

The term aralkyl group generally stands for an aryl group attached to an alkyl group as defined above, such as benzyl or phenethyl.

According to a particular embodiment, A is substituted with at least one substituent, which may be selected from the group consisting in : a hydrogen

atom, a halogen atom (preferably F, Cl, or Br), a hydroxyl group, a (C_1-C_{10})alkyl group, an alkenyl group, an (C_1-C_{10})alkanoyl group, a (C_1-C_{10})alkoxy group, a (C_1-C_{10})alkoxycarbonyl group, an aryl group, an aralkyl group, an arylcarbonyl group, a mono- or poly-cyclic hydrocarbon group, a -NHCO(C_1-C_6)alkyl group, -NO_2, -CN, a $-NR_5R_6$ group or a trifluoro(C_1-C_6)alkyl group, R_5 and R_6, independently from each other, are selected from the group consisting of a hydrogen atom, an alkyl group having from 1 to 10 carbon atoms, an aryl and an aralkyl.

An alkanoyl group is a –CO-alkyl group, the alkyl group being as defined above.

The term arylcarbonyl group generally stands for an aryl group attached to a carbonyl group, the aryl group being as defined above.

The term alkoxycarbonyl group generally stands for an alkoxy group attached to a carbonyl group, the alkoxy group being as defined above.

The term mono- or poly-cyclic hydrocarbon group is understood to refer to hydrocarbon cyclic group having from 1 to 20 carbon atoms, optionally interrupted with one or more heteroatoms selected in the group N, O , S and P. Among such mono- or poly-cyclic hydrocarbon groups, cyclopentyl, cyclohexyl, cycloheptyl, 1- or 2-adamantyl groups, pyran, piperidine, pyrrolidine, morpholine, dioxan, tetrahydrothiophene, and tetrahydrofuran can be cited. The mono- or poly-cyclic hydrocarbon group may form with the phenyl group it is attached an aryl group, such as a α-naphtyl, β-naphtyl, or antracenyl group.

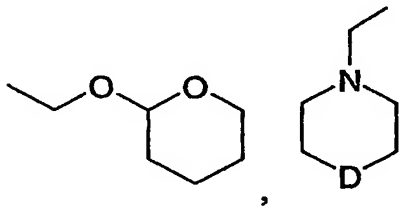
The groups identified above may be optionally substituted. In particular, the alkyl, alkenyl, aryl, aralkyl, and the mono- or poly-cyclic hydrocarbon group may be optionally substituted with one or more groups selected from hydroxyl group, halogen atom, cyano group, nitro group, ester (-COO(C_1-C_6)alkyl group), -OCO(C_1-C_6)alkyl group, amide (-NHCO(C_1-C_6)alkyl or -CONH(C_1-C_6)alkyl group), (C_1-C_{10})alkyl radical, (C_1-C_{10})alkoxy radical, mono- or poly-cyclic

hydrocarbon group, C=O group, a $-NR_5R_6$ group or a trifluoro(C_1-C_6)alkyl group, R_5 and R_6 being as defined above.

The trifluoro(C_1-C_6)alkyl group is preferably the trifluoromethyl group.

According to preferred embodiments, the compounds according to the invention correspond to general formula (I) wherein :

- X is sulfur or preferably oxygen ; and/or
- Y is oxygen ; and/or
- "linker" represents $(CH_2)_n$, wherein n is from 4 to 7 inclusive, or the xylenyl group ; and/or
- R_1 is



, wherein D is oxygen, $-CH_2-$ or NR_3, wherein R_3 preferably represents an alkyl group (said alkyl is more specifically a methyl radical), and-CH_2-B, wherein B is a $-O-CH_2-O-CH_3$ group or $-OSO_2$-alkyl group (wherein alkyl is preferably methyl) or halogen (preferably chlorine); and/or

- R_2 is a hydrogen atom; and/or
- A is a substituted group as defined above.

In a particular embodiment, when A is a substituted group as defined above, at least one of the substituents is an halogen atom, more preferably chlorine or fluorine.

A particular preferred group of compounds according to the present invention, are the compounds of formula (I) wherein at least two substituents simultaneously represent Cl, in particular when A is a naphtalene group.

Another particular preferred group of compounds according to the present invention, are the compounds of formula (I) wherein at least one of the substituents, and more preferably all the substituents, of A represents a hydrogen atom, a methyl group, a propyl group, an ethoxy group, an halogen atom, preferably chlorine or fluorine, or the CF_3 group.

When the compounds according to the invention are in the forms of salts, they are preferably pharmaceutically acceptable salts. Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable base addition salts, pharmaceutically acceptable metal salts, ammonium and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pamoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, EDTA, glycolic, p-aminobenzoic, glutamic, benzenesulfonic, p-toluenesulfonic acids, sulphates, nitrates, phosphates, perchlorates, borates, acetates, benzoates, hydroxynaphthoates, glycerophosphates, ketoglutarates and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2, which is incorporated herein by reference. Examples of metal salts include lithium, sodium, potassium, magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium, tetramethylammonium salts and the like. Examples of organic bases include lysine, arginine, guanidine, diethanolamine, choline and the like.

The pharmaceutically acceptable salts are prepared by reacting the compound of formula I with 1 to 4 equivalents of a base such as sodium hydroxide, sodium methoxide, sodium hydride, potassium t-butoxide, calcium hydroxide, magnesium hydroxide and the like, in solvents like ether, THF, methanol, t-butanol, dioxane, isopropanol, ethanol, etc. Mixture of solvents may be used. Organic bases like lysine, arginine, diethanolamine, choline, guanidine and their derivatives etc. may also be used. Alternatively, acid addition salts wherever applicable are prepared by treatment with acids such as hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, phosphoric acid, p-toluenesulphonic acid, methanesulfonic acid, fonic acid, acetic acid, citric acid, maleic acid, salicylic acid, hydroxynaphthoic acid, ascorbic acid, palmitic acid, succinic acid, benzoic acid, benzenesulfonic acid, tartaric acid and the like in solvents like ethyl acetate, ether, alcohols, acetone, THF, dioxane, etc. Mixture of solvents may also be used.

Specific examples of compounds of formula (I) which fall within the scope of the present invention include the following compounds:

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-pyran-4-one (EHT 3788)

5-[5-(6-Fluoro-2-methyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1593)

5-[5-(6-Fluoro-2-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1074)

5-[5-(7-Propyl-quinolin-8-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 5810)

6,8-Dichloro-4-{5-[4-oxo-6-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-3-yloxy]-pentyloxy}-naphthalene-2-carboxylic acid ethyl ester (EHT 0470)

5-[5-(Benzo[*b*]thiophen-7-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 6060)

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 9376)

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[4-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-butoxy]-4*H*-pyran-4-one (EHT 4745)

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[6-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-hexyloxy]-4*H*-pyran-4-one (EHT 6271)

2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one hydrochloride salt (EHT 1302)

2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 5909)

2-Methoxymethoxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 2168)

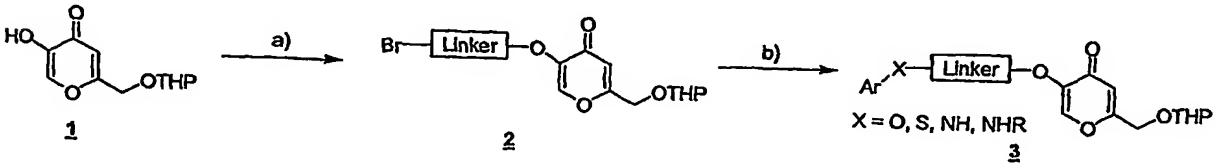
2-Chloromethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 1494)

2-(4-Methyl-piperazin-1-ylmethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7365)

2-Morpholin-4-ylmethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7168)

Particularly preferred compounds are EHT9376 and EHT3788.

The compounds according to the present invention may be prepared by various methods known to those skilled in the art. More preferably, three chemical routes have been carried out. The first one (Scheme 1) relates to compounds included in the structures 3 which can be obtained in two steps starting from compound 1 (described by Miyano, M.; Deason, J. R.; Nakao, A.; Stealey, M. A.; Villamil, C. I.; et al. *J. Med. Chem.* 1988; 31, 1052-1061).

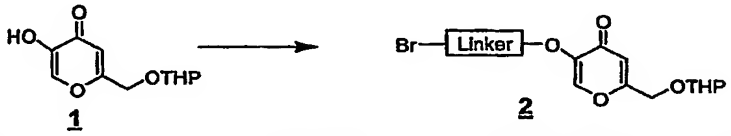


1 2 3

Compound 1 can be treated under alkylation conditions preferably conducted in a solvent, such as DMF or THF, at a temperature between 5°C and 70°C, typically around 80°C using a base such as cesium carbonate (Cs_2CO_3) or NaH

and a dihalogenoalkane or dihalogenoarylalkane (step a) (table 1). The resulting alkylated product 2 can be substituted in a reaction (step b) involving a base such as NaH or cesium carbonate and a nucleophile such 4-quinolinol, 4-quinoline-thiol, 2-quinolinol, 7-benzo[*b*]thiophenol, naphtalen-7-ol non substituted or substituted derivatives or a nucleophile as described in table 2. The preferred solvents are DMF, THF or DMSO and the reaction is conducted at a temperature between 25°C and 100°C.

Table 1



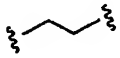
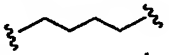
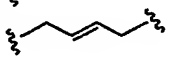
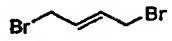
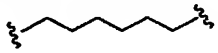
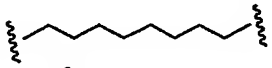
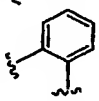
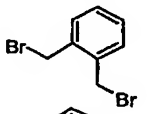
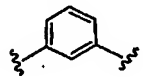
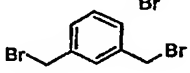
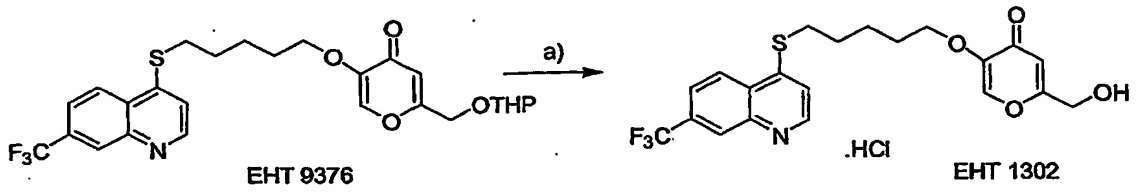
#C's	Linker	Conditions	Alkylating Agent	Yield
2		Cs_2CO_3, DMF, 5°C		16%
3		Cs_2CO_3, DMF, 50°C, 2.5h		66%
4		Cs_2CO_3, DMF, 80°C, 2.5h		71%
4		Cs_2CO_3, DMF, 80°C, 2.5h		12%
5		Cs_2CO_3, DMF, 80°C, 2.5h		95%
6		Cs_2CO_3, DMF, 80°C, 2.5h		23%
7		Cs_2CO_3, DMF, 80°C, 2.5h		79%
8		Cs_2CO_3, DMF, 80°C, 2.5h		62%
4		Cs_2CO_3, DMF, 80°C, 2.5h		11%
5		Cs_2CO_3, DMF, 80°C, 2.5h		22%

Table 2

	#C's	Linker	Conditions	Nucleophile ArXH	Yield
EHT 4745	4		NaH, DMF 25°C	7-trifluoromethyl-4-quinoline-thiol	64%
EHT 9376	5		NaH, THF 25°C	7-trifluoromethyl-4-quinoline-thiol	47%
EHT 6271	6		NaH, DMF 25°C	7-trifluoromethyl-4-quinoline-thiol	33%
EHT 3788	5		NaH, DMF 25°C	7-trifluoromethyl-quinoline-4-ol	18%
EHT 0470	5		NaH, THF 25°C	6,8-dichloro-4-hydroxy-naphtalene-2-carboxylic acid ethyl ester	17%
EHT 1074	5		NaH, DMSO, 60°C	6-fluoro-2-trifluoromethyl-quinolin-4-ol	12%
EHT 1593	5		NaH, DMSO, 60°C	6-fluoro-2-methyl-quin-4-ol	20%
EHT 6060	5		NaH, THF 25°C	benzo[b]thiophen-7-ol	3%

The second preferred chemical route (Scheme 2) uses the EHT 9376 compound which can be deprotected to the corresponding alcohol EHT 1302 in methanol with an acid source (MeOH, HCl) such as Dowex resin 50WX8-200 at a temperature comprised between 5°C and 30°C, typically around 25°C.

Scheme 2:



EHT 9376 EHT 1302

In the third preferred chemical route (Scheme 3) Intermediate 5 can be prepared from the silylated ether 4 (Sefkow, M.; Kaatz, H. *Tetrahedron Lett*, 1999, *40*, 6561-6562) with a base such as cesium carbonate and 1,5-dibromopentane in dimethylformamide at 50°C (Scheme 3).

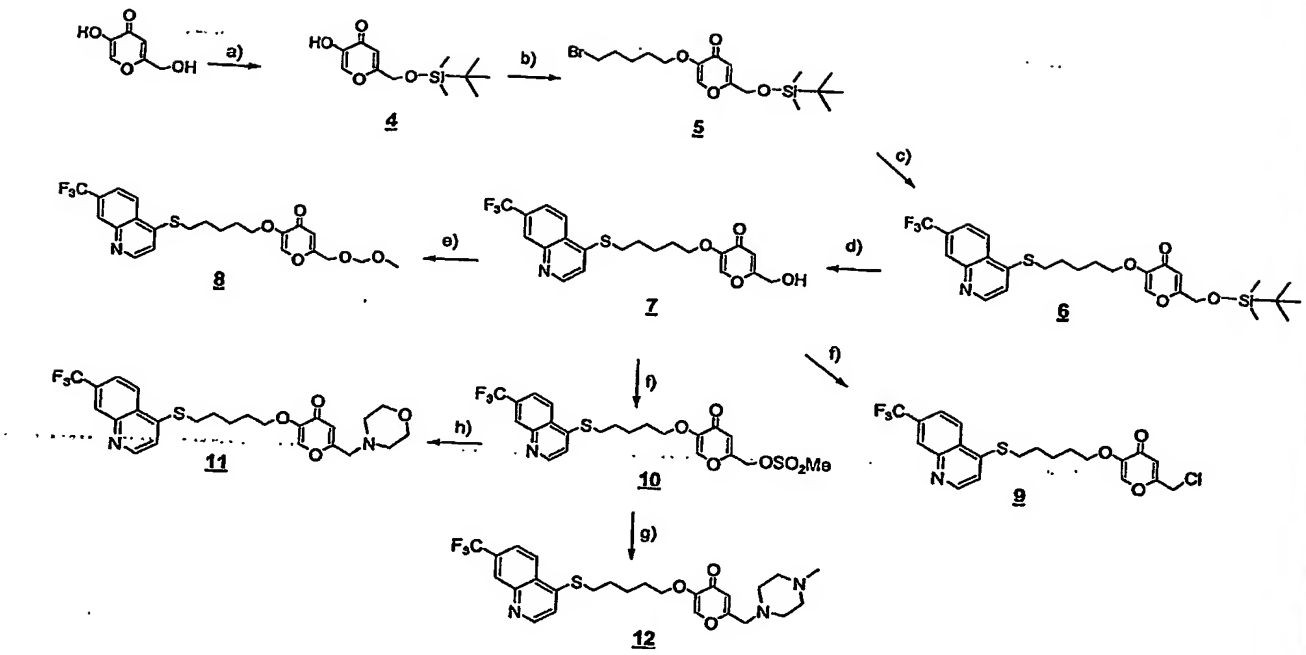
Derivative 6 can be prepared from intermediate 4 using sodium hydride, 7-trifluoromethyl-4-quinoline-thiol in dimethylformamide at room temperature. Subsequent deprotection of silylated ether 6 using *n*-tetrabutylammonium fluoride in tetrahydrofuran at room temperature led to alcohol 7 (scheme 3).

MOM derivative 8 can be obtained from alcohol 7 with sodium hydride, and methylchloromethyl ether in tetrahydrofuran at room temperature.

Chloro and mesylate derivatives 9 and 10 can be prepared from alcohol 7 using methanesulfonyl chloride and triethylamine in dichloromethane at room temperature.

Finally morpholino and *N*-methylpiperazine derivative 11 and 12 can be obtained from mesylate 10 using respectively morpholine or *N*-methylpiperazine in dichloromethane at reflux (scheme 3).

Scheme 3



a) TBDMSCl, CH_2Cl_2, TEA, RT. b) Cs_2CO_3, DMF, dibromopentane, 50°C c) 7-trifluoromethyl-4-quinoline-thiol, NaH, DMF, RT. d) TBAF, THF, RT e) MOMCl, NaH, THF, RT. f) CH_3SO_2Cl, TEA, CH_2Cl_2, RT. g) *N*-methylpiperazine, CH_2Cl_2, reflux. h) Morpholine, CH_2Cl_2, reflux.

For the above described series, yields are generally comprised between 55 and 90% by weight, more specifically between 55 and 75% by weight. These methods for preparing compounds of formula (I) represent further objects of the present application.

It should be understood that other ways of producing these compounds may be designed by the skilled person, based on common general knowledge and following guidance contained in this application.

As indicated above, a further object of this invention relates to a pharmaceutical composition comprising at least one compound of formula (I), as defined above, and a pharmaceutically acceptable vehicle or support.

The compounds may be formulated in various forms, including solid and liquid forms, such as tablets, gel, syrup, powder, aerosol, etc.

The compositions of this invention may contain physiologically acceptable diluents, fillers, lubricants, excipients, solvents, binders, stabilizers, and the like. Diluents that may be used in the compositions include but are not limited to dicalcium phosphate, calcium sulphate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch, powdered sugar and for prolonged release tablet-hydroxy propyl methyl cellulose (HPMC). The binders that may be used in the compositions include but are not limited to starch, gelatin and fillers such as sucrose, glucose, dextrose and lactose.

Natural and synthetic gums that may be used in the compositions include but are not limited to sodium alginate, ghatti gum, carboxymethyl cellulose, methyl cellulose, polyvinyl pyrrolidone and veegum. Excipients that may be used in the compositions include but are not limited to microcrystalline cellulose, calcium sulfate, dicalcium phosphate, starch, magnesium stearate, lactose, and sucrose. Stabilizers that may be used include but are not limited to polysaccharides such as acacia, agar, alginic acid, guar gum and tragacanth, amphotsics such as gelatin and synthetic and semi-synthetic polymers such as carbomer resins, cellulose ethers and carboxymethyl chitin.

Solvents that may be used include but are not limited to Ringers solution, water, distilled water, dimethyl sulfoxide to 50% in water, propylene glycol (neat or in water), phosphate buffered saline, balanced salt solution, glycol and other conventional fluids.

The dosages and dosage regimen in which the compounds of formula (I) are administered will vary according to the dosage form, mode of administration, the condition being treated and particulars of the patient being treated. Accordingly, optimal therapeutic concentrations will be best determined at the time and place through routine experimentation.

The compounds according to the invention can also be used enterally. Orally, the compounds according to the invention are suitable administered at the rate of 100 µg to 100 mg per day per kg of body weight. The required dose can be administered in one or more portions. For oral administration, suitable forms are,

for example, tablets, gel, aerosols, pills, dragees, syrups, suspensions, emulsions, solutions, powders and granules; a preferred method of administration consists in using a suitable form containing from 1 mg to about 500 mg of active substance.

The compounds according to the invention can also be administered parenterally in the form of solutions or suspensions for intravenous or intramuscular perfusions or injections. In that case, the compounds according to the invention are generally administered at the rate of about 10 µg to 10 mg per day per kg of body weight; a preferred method of administration consists of using solutions or suspensions containing approximately from 0.01 mg to 1 mg of active substance per ml.

The compounds of formula (I) can be used in a substantially similar manner to other known anti-tumor agents for treating (both chemopreventively and therapeutically) various tumors or to other known agents for treating ocular diseases and especially anti-retinopathic or anti-arthritis agents. For the compounds of this invention, the dose to be administered, whether a single dose, multiple dose, or a daily dose, will of course vary with the particular compound employed because of the varying potency of the compound, the chosen route of administration, the size of the recipient, the type of disease (cancer, and in such a case the type of tumor, arthritis, ocular-diseases and especially retinopathies, and in such a case the type of retinopathy, especially diabetic retinopathy, retinal degenerative diseases, Age-Related Macular Degeneration (ARMD)) and the nature of the patient's condition. The dosage to be administered is not subject to definite bounds, but it will usually be an effective amount, or the equivalent on a molar basis of the pharmacologically active free form produced from a dosage formulation upon the metabolic release of the active drug to achieve its desired pharmacological and physiological effects. An oncologist skilled in the art of cancer treatment or a doctor skilled in the art for ocular-diseases, diabetes or arthritis treatment will be able to ascertain, without undue experimentation, appropriate protocols for the effective administration of the compounds of this present invention, such as by referring to the earlier published studies on

compounds found to have anti-tumor, anti-angiogenic and especially anti-retinopathic or anti-arthritis properties.

According to another aspect, the present invention relates to a method for the treatment of a disease associated with abnormal cell proliferation, comprising administering to a patient in need of such treatment an effective amount of at least one compound of general formula (I) as described above.

Preferred compounds for use according to the invention include any sub-group as defined above, and, as specific examples, the following compounds:

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-pyran-4-one (EHT 3788)
5-[5-(6-Fluoro-2-methyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1593)
5-[5-(6-Fluoro-2-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1074)
5-[5-(7-Propyl-quinolin-8-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 5810)
6,8-Dichloro-4-{5-[4-oxo-6-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-3-yloxy]-pentyloxy}-naphthalene-2-carboxylic acid ethyl ester (EHT 0470)
5-[5-(Benzo[*b*]thiophen-7-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 6060)
2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 9376)
2-(Tetrahydro-pyran-2-yloxymethyl)-5-[4-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-butoxy]-4*H*-pyran-4-one (EHT 4745)
2-(Tetrahydro-pyran-2-yloxymethyl)-5-[6-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-hexyloxy]-4*H*-pyran-4-one (EHT 6271)
2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one hydrochloride salt (EHT 1302)

2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 5909)

2-Methoxymethoxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 2168)

2-Chloromethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 1494)

2-(4-Methyl-piperazin-1-ylmethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7365)

2-Morpholin-4-ylmethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7168)

A further object of this invention is the use of an effective amount of at least one compound of formula (I) as defined above for the preparation of pharmaceutical composition for the treatment of a disease associated with abnormal cell proliferation and of a disease associated with unregulated angiogenesis.

Because of their cell proliferation inhibitory activity, the compounds of this invention are suitable for treating a variety of diseases in a variety of conditions. In this regard, "treatment" or "treating" include both therapeutic and prophylactic treatments. Accordingly, the compounds may be used at very early stages of a disease, or before early onset, or after significant progression, including metastasis. The term "treatment" or "treating" designates in particular a reduction of the burden in a patient, such as a reduction in cell proliferation rate, a destruction of diseased proliferative cells, an inhibition of the formation of vessel or vessel-like structure, a reduction of tumor mass or tumor size, a delaying of tumor progression, as well as a complete tumor suppression.

Typical examples of diseases associated with abnormal cell proliferation and/or with unregulated angiogenesis include cancers and restenosis, arthritis, diabetes, ocular-diseases and especially retinopathies, for instance. The compounds of this invention are particularly suited for the treatment of cancers, such as solid tumors or lymphoid tumors. Specific examples include prostate

cancer, ovarian cancer, pancreas cancer, lung cancer, breast cancer, liver cancer, head and neck cancer, colon cancer, bladder cancer, non-Hodgkin 's lymphoma cancer and melanoma.

The compounds may be administered according to various routes, typically by injection, such as local or systemic injection(s). Intratumoral injections are preferred for treating existing cancers. However, other administration routes may be used as well, such as intramuscular, intravenous, intradermic, subcutaneous, etc. Furthermore, repeated injections may be performed, if needed, although it is believed that limited injections will be needed in view of the efficacy of the compounds.

A further object of this invention is a composition for reducing cancer cell proliferation by administering in a subject having cancer an effective amount of compound of formula (I) as defined above.

A further object of this invention is a composition for treating metastatic cancers by administering in a subject in need of such treatment an effective amount of compound of formula (I) as defined above.

A further object of this invention is a composition for treating a eye-disease by administering in a subject in need of such treatment an effective amount of compound of formula (I) as defined above.

A further object of this invention is a composition for treating arthritis by administering in a subject in need of such treatment an effective amount of compound of formula (I) as defined above.

A further object of this invention is the use of a compound as defined above for the preparation of a pharmaceutical composition for treating metastatic cancers or for reducing cancer cell proliferation.

A further object of this invention is the use of a compound as defined above for the preparation of a pharmaceutical composition for treating an ocular-disease, especially retinopathies (including diabetic retinopathies, retinal degenerative diseases and Age-Related Macular Degeneration (ARMD)).

A further object of this invention is the use of a compound as defined above for the preparation of a pharmaceutical composition for treating arthritis.

Further aspects and advantages of this invention will be disclosed in the following examples, which should be regarded as illustrative and not limiting the scope of this application.

LEGEND TO THE FIGURES

Figure 1: Examples of dose-response curves for anchorage-independent growth assays in soft agar.Example of a cystostatic compound (L651582, left) and of a cytotoxic compound (EHT 8617, right).

Figure 2: Antiproliferative effect of compounds on HCT116 (top) and HMEC1 (bottom) cell lines measured by MTT viability assay.
2.5 10^3 (HCT116) or 5 10^4 (HMEC1) cells were seeded in 48-well plates in growth medium containing 10% FBS, with or without various concentrations of test compounds. Cell cultures were fed every 3 days with the appropriate media. Cell viability was determined on day 6. Data were analyzed and IC50s were calculated from the dose-response curves using GraphPad Prism. Results displayed on the graph are mean $\pm$ SEM of 1 to 3 experiments

Figure 3: Effect of the treatment of HCT116 cells with the compounds on the size of the clones grown in soft agar.
5 10^3 cells were seeded in 24-well plates in 0.3% agar-containing medium supplemented with the designated amount of compounds. After 7 days of incubation at 37°C, pictures were taken of each well and were analyzed using

the ImageJ image analysis software. In particular, clone size and number were calculated. The data were analyzed using GraphPad Prism, and IC50 was calculated. Results displayed on the graph are mean ± SEM of 1 to 3 experiments.

Figure 4: Dose response curves for HCT116 cells grown in soft agar (clone number/clone size).

Figure 5: Anti-proliferative effect of EHT 9376 and its reversibility on HCT116 cells measured by MTT.
Cells were grown with medium with or without 1 µM L651582 (left) or EHT 9376 (right). After 3 days of treatment, the compound medium in one set of cells was removed and replaced with regular growth medium. On the designated days, the number of viable cells was determined by the MTT cell proliferation assay. Results were normalized against the untreated control. **highest line:** untreated, **medium line:** compound removal at day 3, **lowest line:** 7-day treatment. Values are the mean ± SEM of duplicate experiments.

Figure 6: Migration of MDA-MB-231 cells in the presence of various concentrations of L651582, and EHT 9376.
5 10^4 MDA-MB-231 cells, resuspended in culture medium with or without Fetal Bovine Serum (FBS), were seeded in the upper Boyden blind well on top of 8 µm pore-sized filters. The ability of cells to migrate through the filter was assayed in the absence or presence of FBS in the lower Boyden well. After incubation at 37°C for 16 hours, the medium was removed and replaced with calcein containing medium. After labelling, cells were washed and resuspended in HBSS and fluorescence was read in a fluoroskan. Fluorescence values were normalized against the fluorescence obtained for the 1% DMSO control. The data plotted are the means ± SEM for 2 wells under the different conditions.

Figure 7: Influence of various compounds on *in vitro* tubule formation.

HMEC1 cells were plated on Matrigel-coated wells as described in the material and methods. Cultures were photographed after 7 hours of incubation. The "total tubule length" and "number of junction" parameters were quantitated using the AngioSys software and data were analyzed using GraphPad Prism for IC50 calculation. The ratios IC50 L651582/IC50 compound were calculated and plotted in the above bar graphs. Values represent the mean ± SEM of 2 to 8 experiments performed in duplicate.

EXAMPLES

Examples 1 to 20 disclose the synthesis and physico-chemical properties of compounds according to this invention.

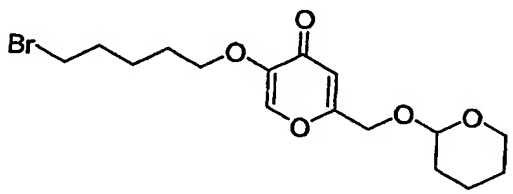
Example 21 discloses the biological activity of the compounds.

Preparation of EHT 3788, 1593, EHT 1074, EHT 5810, EHT 0470, EHT 6060 and EHT 9376.

Example 1: 5-(5-Bromo-pentyloxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one 1.

The compound was prepared according to the above general procedure using 5-hydroxy-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (3.5 g, 15.5 mmol) in 20 mL of DMF, Cs_2CO_3 (5.04 g, 15.5 mmol) and 1,5-dibromopentane (8.8 g, 36.7 mmol). The sealed tube was heated at 90-95 °C for 1 h 40. A white solid 1 was obtained (5.30 g, 91 % yield).

The structure of compound ex 1 is presented below:



MW: 375.25; Yield: 91 %; Yellow solid; Mp: 140.3 °C.

R_f: 0.36 (CH_2Cl_2:ethyl acetate = 8:2).

^{1}H-NMR ($CDCl_3$, δ): 1.53-1.84 (m, 12H, 6xCH_2), 3.52-3.57 (m, 1H, OCH_2), 3.77-3.84 (m, 1H, O-CH_2), 4.30 (d, J_{BA} = 14.5 Hz, 1H, OCH_2), 4.48 (s, 2H, OCH_2), 4.50 (d, J_{AB} = 14.5 Hz, 1H, OCH_2), 4.70 (t, J = 3.1 Hz, 1H, OCHO), 5.07 (s, 2H, $BrCH_2$), 6.51 (s, 1H, –C=CH-), 7.36-7.42 (m, 4H, Ar-H), 7.53 (s, 1H, --C=CH-).

MS-ESI *m/z* (rel. int.): 374.9-376.9 ($[MH]^+$, 100).

HPLC: Method A, Detection UV 254 nm, RT= 5.73 min.

General procedures.

Method A (in THF):

In a 25 mL round-bottom flask equipped with a magnetic stirrer and under an inert atmosphere were charged successively one equivalent of NaH (60 % in mineral oil), anhydrous THF (10 mL) and the monomer to be deprotonated (250 mg). The reaction mixture was abandoned until no evolution of gas was observed (between 3 and 5 hours). A solution 1 M of 5-(5-bromo-pentyloxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one **1** in THF (1 eq) was added and the reaction mixture was stirred 12 h at room temperature. The reaction mixture is evaporated *in vacuo*, the crude product is purified by a wash with a solution of aqueous NaOH 2N and/or by flaschromatography on silica.

Method B (in DMSO):

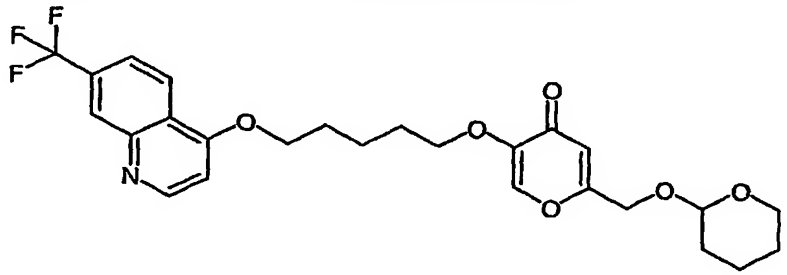
In a 50 mL round-bottom flask equipped with a magnetic stirrer and under an inert atmosphere were charged successively one equivalent of NaH (60 % in mineral oil), DMSO (5 mL) and the monomer to be deprotonated (250 mg). The reaction mixture was heated at 60 °C for 3 hours. After cooling to room temperature, the 5-(5-bromo-pentyloxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one **1** (1 eq) was added (in one time) and the reaction mixture was heated at 60 °C for 12 h. After cooling, 50 mL of dichloromethane was added,

the organic layer is washed with H_2O (4 x 10 mL), dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude product is purified by a wash with a solution of aqueous NaOH 2N and/or by flaschromatography on silica.

Example 2a: 2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-pyran-4-one (EHT 3788).

The compound was prepared according to method A with 7-trifluoromethyl-quinolin-4-ol (0.25 g, 0.12 mmol). After purification by chromatography on silica using as eluent CH_2Cl_2:MeOH = 95:5 a beige solid **EHT 3788** (0.11 g, 18 % yield) was obtained.

The structure of compound ex 2a is presented below:



MW: 507.50; Yield 18 %; beige solid.

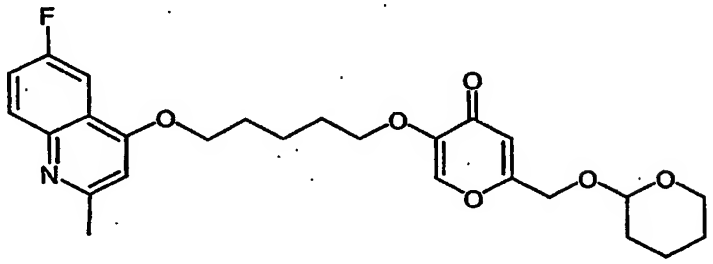
^{1}H-NMR ($CDCl_3$, δ): 1.50-2.12 (m, 12H, 6xCH_2), 3.50-3.58 (m, 1H, CH_2CH_2O), 3.75-3.85 (m, 1H, CH_2CH_2O), 3.96 (t, J = 7.0 Hz, 2H, OCH_2), 4.27 (t, J = 7.0 Hz, 2H, OCH_2), 4.34 (dd, J_{BA} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 4.52 (dd, J_{AB} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 4.73 (m, 1H, OCHO), 6.52 (d, J = 0.6 Hz, 1H, -C=CH-), 6.83 (d, J = 5.7 Hz, 1H, Ar-H), 7.59 (s, 1H, -C=CH-), 7.68 (d, J = 9.6 Hz, 1H, Ar-H), 8.34 (m, 1H, Ar-H), 8.83 (dd, J = 6.3 Hz, J = 5.6 Hz, 1H, Ar-H).

Example b: 5-[5-(6-Fluoro-2-methyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1593).

The compound was prepared according to method A with 6-fluoro-2-methyl-quinolin-4-ol (0.25 g, 1.41 mmol). After purification by chromatography on silica

using as eluent heptane:AcOEt = 9:1 a brown oil **EHT 1593** (0.13 g, 19.5 % yield) was obtained.

The structure of compound ex 2b is presented below:



MW: 471.52; Yield 19.5 %; Brown oil.

^{1}H-NMR ($CDCl_3$, δ): 1.50-2.02 (m, 12H, 6xCH_2), 2.69 (s, 3H, CH_3), 3.50-3.58 (m, 1H, CH_2CH_2O), 3.75-3.85 (m, 1H, CH_2CH_2O), 3.68 (t, J = 6.3 Hz, 2H, OCH_2), 4.21 (t, J = 6.3 Hz, 2H, OCH_2), 4.34 (dd, J_{BA} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 4.53 (dd, J_{AB} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 5.32 (m, 1H, OCHO), 6.52 (s, 1H, -C=CH-), 6.63 (s, 1H, Ar-H), 7.38-7.46 (m, 1H, Ar-H), 7.60 (s, 1H, -C=CH-), 7.74 (dd, J = 6.3 Hz, J = 2.7 Hz, 1H, Ar-H), 7.93 (dd, J = 6.3 Hz, J = 5.1 Hz, 1H, Ar-H).

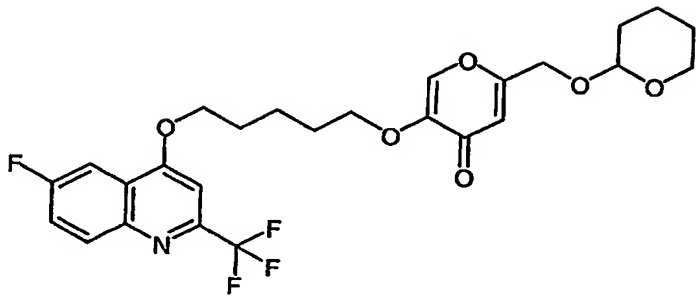
MS-ESI *m/z* (rel. int.): 472.1 ($[MH]^+$, 90), 388.0 (15), 295.1 (100), 227.0 (15), 211.1 (10), 178.0 (45).

HPLC: Method A, detection UV 254 nm, **EHT 1593** RT = 4.62 min, peak area 95.7%

Exemple 3: 5-[5-(6-Fluoro-2-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1074).

The compound was prepared according to method B with 6-fluoro-2-trifluoromethyl-quinolin-4-ol (0.25 g, 1.08 mmol). After purification by chromatography on silica using as eluent CH_2Cl_2:MeOH = 98:2 a grey solid **EHT 1074** (0.07 g, 12 % yield) was obtained.

The structure of compound ex 3 is presented below:



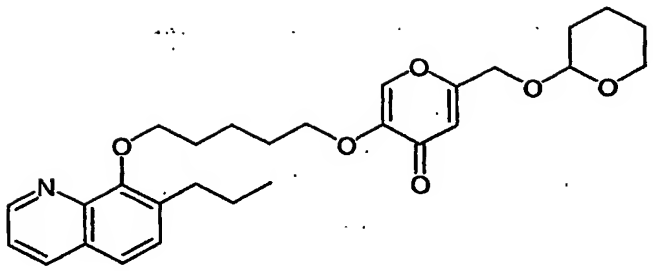
MW: 525.49; Yield: 12 %; grey solid.

^{1}H-NMR (CD_3Cl, δ): 1.50-1.92 (m, 8H, 4xCH_2), 1.92-2.12 (m, 4H, 2xCH_2), 3.52-3.59 (m, 1H, CH_2O), 3.83-3.90 (m, 1H, CH_2O), 3.97 (t, J = 6.3 Hz, 2H, OCH_2O), 4.32 (t, J = 5.7 Hz, 2H, OCH_2O), 4.34 (dd, J_{BA} = 14.4 Hz, J = 0.6 Hz, 1H, =CCH_2O), 4.53 (dd, J_{AB} = 14.4 Hz, J = 0.6 Hz, 1H, =CCH_2O), 4.72 (t, J = 3.0 Hz, 1H, OCH_2O), 6.52 (d, J = 0.6 Hz, 1H, –CH=), 7.06 (s, 1H, Ar-H), 7.56 (m, 1H, Ar-H), 7.61 (s, 1H, –CH=), 7.85 (dd, J = 9.3 Hz, J = 3.0 Hz, 1H, Ar-H), 8.16 (dd, J = 9.3 Hz, J = 5.1 Hz, 1H, Ar-H).

Exemple 4: 5-[5-(7-Propyl-quinolin-8-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 5810).

The compound was prepared according to method B with 7-propyl-quinolin-8-ol (0.25 g, 1.33 mmol). After purification by chromatography on silica using as eluent CH_2Cl_2:MeOH = 98:2 a green oil **EHT 5810** (0.16 g, 25 % yield) was obtained.

The structure of compound ex 4 is presented below:



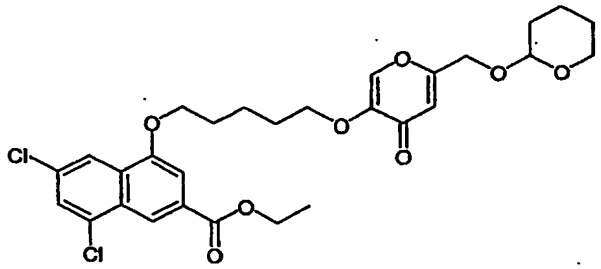
MW: 481.58; Yield: 25 %; Green oil.

^{1}H-NMR (CD_3Cl, δ): 1.00 (t, *J* = 7.2 Hz, 3H, Me), 1.50-1.92 (m, 10H, 5xCH_2), 1.92-2.10 (m, 4H, 2xCH_2), 2.84 (t, *J* = 7.2 Hz, 2H, =C-*C*$H_2$$CH_2CH_3$), 3.52-3.59 (m, 1H, CH_2O), 3.83-3.90 (m, 1H, CH_2O), 3.94 (t, *J* = 6.6 Hz, 2H, OCH_2O), 4.32 (t, *J* = 6.6 Hz, 2H, OCH_2O), 4.34 (dd, J_{BA} = 14.4 Hz, *J* = 0.6 Hz, 1H, =CCH_2O), 4.52 (dd, J_{AB} = 14.4 Hz, *J* = 0.6 Hz, 1H, =CCH_2O), 4.73 (t, *J* = 3.0 Hz, 1H, OCH_2O), 6.52 (d, *J* = 0.6 Hz, 1H, –C=CH-), 7.33 (dd, *J* = 8.1 Hz, *J* = 4.2 Hz, 1H, Ar-H), 7.38 (d, J_{BA} = 8.4 Hz, 1H, Ar-H), 7.50 (d, J_{BA} = 8.4 Hz, 1H, Ar-H), 7.59 (s, 1H, –C=CH-), 8.09 (d, *J* = 1.8 Hz, 1H, Ar-H), 8.16 (dd, *J* = 4.2 Hz, *J* = 1.8 Hz, 1H, Ar-H).

Exemple 5: 6,8-Dichloro-4-{5-[4-oxo-6-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-3-yloxy]-pentyloxy}-naphthalene-2-carboxylic acid ethyl ester (EHT 0470).

The compound was prepared according to method A with 6,8-dichloro-4-hydroxy-naphthalene-2-carboxylic acid ethyl ester (0.25 g, 0.88 mmol). After purification by chromatography on silica using as eluent CH_2Cl_2:MeOH = 98:2 an amber oil **EHT 0470** (0.085 g, 17 % yield) was obtained.

The structure of compound ex 5 is presented below:



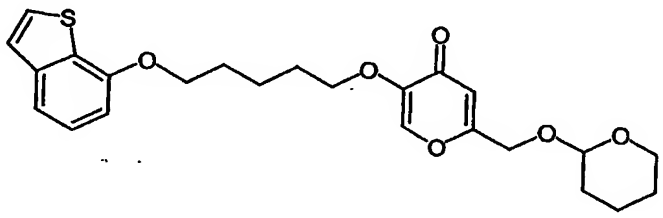
MW: 579.47; Yield: 17 %; Amber oil.

^{1}H-NMR (CD_3Cl, δ): 1.47 (t, J = 7.2 Hz, 3H, Me), 1.50-1.92 (m, 8H, 4xCH_2), 1.92-2.10 (m, 4H, 2xCH_2), 3.52-3.59 (m, 1H, CH_2O), 3.83-3.90 (m, 1H, CH_2O), 3.96 (t, J = 6.3 Hz, 2H, OCH_2O), 4.23 (t, J = 6.3 Hz, 2H, OCH_2O), 4.34 (dd, J_{BA} = 14.4 Hz, J = 0.6 Hz, 1H, =CCH_2O), 4.47 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.53 (dd, J_{AB} = 14.4 Hz, J = 0.6 Hz, 1H, =CCH_2O), 4.73 (t, J = 3.0 Hz, 1H, OCH_2O), 6.52 (d, J = 0.6 Hz, 1H, –C=CH-), 7.46 (d, J = 1.2 Hz, 1H, Ar-H), 7.60 (s, 1H, –C=CH-), 7.62 (d, J = 2.1 Hz, 1H, Ar-H), 8.19 (dd, J = 2.1 Hz, J = 0.6 Hz, 1H, Ar-H), 8.54 (d, J = 1.2 Hz, 1H, Ar-H).

Exemple 6: 5-[5-(Benzo[*b*]thiophen-7-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 6060).

The compound was prepared according to method A with benzo[*b*]thiophen-7-ol (0.25 g, 1.66 mmol). After purification by chromatography on silica using as eluent CH_2Cl_2:MeOH = 95:5 a yellow oil **EHT 6060** (0.02 g, 3 % yield) was obtained.

The structure of compound ex 6 is presented below:



MW: 444.54; Yield: 3 %; Yellow oil.

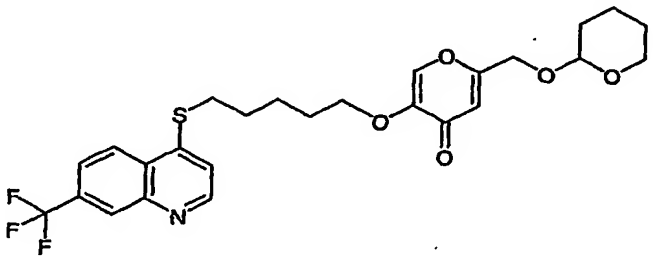
^{1}H-NMR ($CDCl_3$, δ): 1.50-2.00 (m, 12H, 6xCH_2), 3.52-3.60 (m, 1H, CH_2CH_2O), 3.82-3.90 (m, 1H, CH_2CH_2O), 3.94 (t, 2H, J = 6.6 Hz, OCH_2), 4.27 (t, J = 6.3 Hz, 2H, OCH_2), 4.34 (dd, J_{BA} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 4.64 (dd, J_{AB} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 4.72-4.77 (m, 1H, OCHO), 6.52 (d, J = 0.6 Hz, 1H, -

C=CH-), 6.70 (d, J = 5.4 Hz, 1H, Ar-H), 7.53 (d, J = 5.4 Hz, 1H, Ar-H), 7.59 (s, 1H, -C=CH-), 7.68 (d, J = 5.4 Hz, 1H, Ar-H), 8.57 (d, J = 5.4 Hz, 1H, Ar-H).

Exemple 7: 2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 9376).

The compound was prepared according to method A with 7-trifluoromethyl-quinoline-4-thiol (0.25 g, 1.09 mmol). After purification by chromatography on silica using as eluent CH_2Cl_2:MeOH = 99:1 a yellow solid **EHT 9376** (0.27 g, 47 % yield) was obtained.

The structure of compound ex 7 is presented below:



MW: 523.57, Yield: 47 %; Yellow solid; Mp = 86.4 °C.

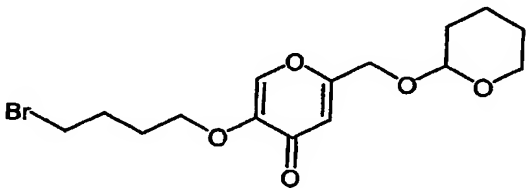
^{1}H-NMR (CD_3Cl, δ): 1.49-1.90 (m, 12H, 6xCH_2), 3.16 (t, J = 7.2 Hz, 2H, CH_2S), 3.50-3.58 (m, 1H, CH_2O), 3.83-3.91 (m, 1H, CH_2O), 3.91 (t, J = 6.3 Hz, 2H, CH_2O), 4.33 (dd, J_{BA} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 4.52 (dd, J_{BA} = 14.4 Hz, J = 0.6 Hz, 1H, CH_2O), 4.73 (m, 1H, OCHO), 6.52 (s, 1H, –C=CH-), 7.28 (d, J = 4.8 Hz, 1H, Ar-H), 7.58 (s, 1H, –C=CH-), 7.72 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H, Ar-H), 8.25 (d, J = 8.7 Hz, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.80 (d, J = 4.8 Hz, 1H, Ar-H).

Synthesis of EHT 4745, EHT 6271, EHT 1302.

Exemple 8: 5-(4-Bromo-butoxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one 2.

The compound was prepared according to the above general procedure using 5-hydroxy-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (1.00 g, 4.42 mmol), Cs_2CO_3 (1.58 g, 4.86 mmol) and 1,4-dibromobutane (2.00 mL, 16.7 mmol). The sealed tube was heated at 80°C for 2 h 30. A white solid **2** was obtained (1.14 g, 71 % yield).

The structure of compound ex 8 is presented below:



MW: 361.23; Yield: 71 %, White solid, Mp = 71.5 °C.

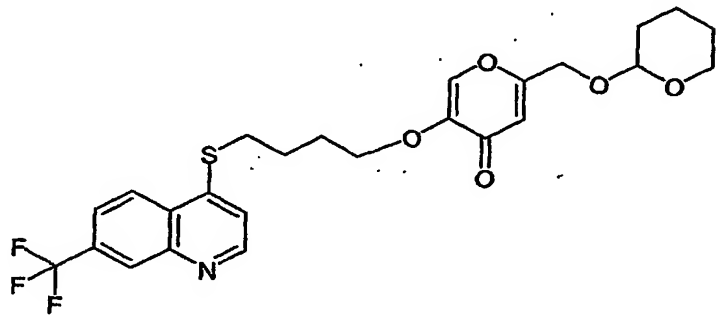
^{1}H-NMR ($CDCl_3$, δ): 1.49-1.91 (m, 6H, 3xCH_2), 1.91-2.10 (m, 4H, 2xCH_2), 3.48 (t, J = 6.5 Hz, 2H, BrCH_2), 3.50-3.59 (m, 1H, OCH_2), 3.76-3.88 (m, 1H, OCH_2), 3.92 (t, J = 6.1 Hz, 2H, OCH_2), 4.32 (d, J_{BA} = 14.4 Hz, 1H, =CCH_2O), 4.51 (d, J_{AB} = 14.4 Hz, 1H, =CCH_2O), 4.70-4.75 (m, 1H, OC*H*O), 6.50 (s, 1H, -C=CH-), 7.58 (s, 1H, -C=CH-).

Exemple 9: 2-(Tetrahydro-pyran-2-yloxymethyl)-5-[4-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-butoxy]-4*H*-pyran-4-one (EHT 4745).

7-Trifluoromethyl-4-quinoline-thiol (0.19 g, 0.87 mmol) was charged in a 25 mL round-bottomed flask equipped with a magnetic stirrer and under inert atmosphere. Anhydrous DMF (10 mL) and NaH 60% dispersion in oil (35 mg, 0.87 mmol) were successively added. After 30 min a solution of 5-(4-bromo-butoxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one **2** (0.30 g, 0.83 mmol) was added at room temperature. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was poured in 200 mL of H_2O, extracted with AcOEt (3 x 75 mL). The organic layer was washed with brine (4 x

250 mL), dried over $MgSO_4$, filtered and evaporated to dryness. The crude compound was recrystallized in diethyl ether to give after filtration **EHT 4745** (271 mg, 64 % yield) as a yellow solid.

The structure of compound ex 9 is presented below:



MW: 508.55; Yield: 64 %; Yellow pale solid; Mp: 97.3 °C (dec.).

R_f: 0.12 (AcOEt).

^{1}H-NMR (CD_3Cl, δ): 1.45-1.92 (m, 6H, 3xCH_2), 1.98-2.12 (m, 4H, 2xCH_2), 3.19-3.28 (t, J = 6.8 Hz, 2H, CH_2S), 3.52-3.59 (m, 1H, CH_2O), 3.92-4.01 (m, 1H, CH_2O), 4.32 (dd, J_{BA} = 14.4 Hz, J = 0.5 Hz, 1H, =CCH_2O), 4.52 (dd, J_{AB} = 14.4 Hz, J = 0.5 Hz, 1H, =CCH_2O), 4.72 (t, J = 3.1 Hz, 2H, OCH_2O), 6.52 (s, 1H, –C=CH-), 7.32 (d, J = 4.8 Hz ,1H, Ar-H), 7.59 (s, 1H, –C=CH-), 7.71 (dd, J = 8.8 Hz, J = 1.7 Hz, 1H, Ar-H), 8.24 (d, J = 8.8 Hz, 1H, Ar-H), 8.36 (s, 1H, Ar-H), 8.80 (d, J = 4.8 Hz, 1H, Ar-H).

MS-ESI *m/z* (rel. int.): 510 ([MH]$^+$, 30), 426 (100).

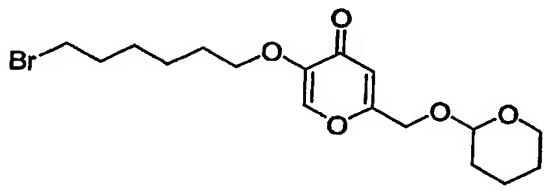
HPLC: Method A, detection UV 254 nm, **EHT 4745** RT = 5.33 min, peak area 99.9 %.

Exemple 10: 5-(5-Bromo-hexyloxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one 3.

5-Hydroxy-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (1.50 g, 6.60 mmol) was charged in a 30 mL sealed tube equipped with a magnetic stirrer and

under inert atmosphere. Anhydrous DMF (10 mL), Cs_2CO_3 (2.30 g, 7.00 mmol) and 1,6-dibromo-hexane (3.20 g, 13.30 mmol) were successively added. The reaction mixture was stirred 2 h at 60°C. After evaporation of DMF, the crude compound was purified by column chromatography (SiO_2, CH_2Cl_2:AcOEt = 8:2) to give after evaporation 5-(6-bromo-hexyloxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one **3** as an oil (190 mg, 52 % yield).

The structure of compound ex 10 is presented below:



MW: 389.28; Yield: 52%; Oil.

^{1}H-NMR ($CDCl_3$, δ): 1.40-1.95 (m, 14H, 7xCH_2), 3.45 (t, J = 6.7 Hz, 2H, BrCH_2), 3.52-3.64 (m, 1H, OCH_2), 3.82-3.92 (m, 1H, OCH_2), 3.90 (t, J = 6.5 Hz, 2H, OCH_2), 4.36 (d, J_{BA} = 14.4 Hz, 1H, =CCH_2O), 4.56 (d, J_{AB} = 14.4 Hz, 1H, =CCH_2O), 4.74-4.79 (m, 1H, OC*H*O), 6.54 (s, 1H, -C=CH-), 7.60 (s, 1H, -C=CH-).

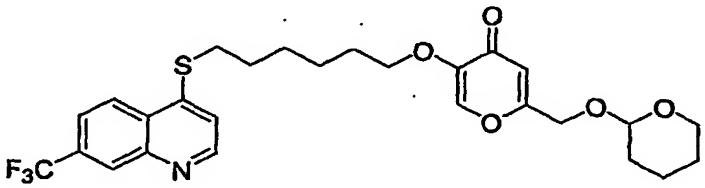
MS-ESI *m/z* (rel. int.): 389-391 ($[MH]^+$, 97-100).

Exemple 11: 2-(Tetrahydro-pyran-2-yloxymethyl)-5-[6-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-hexyloxy]-4*H*-pyran-4-one (EHT 6271).

7-Trifluoromethyl-4-quinoline-thiol (0.62 g, 2.70 mmol) was charged in a 50 mL round-bottomed flask equipped with a magnetic stirrer and under inert atmosphere. Anhydrous DMF (20 mL) and NaH 60% dispersion in oil (110 mg, 2.70 mmol) were successively added. After 30 min a solution of 5-(6-bromo-hexyloxy)-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one **3** (1.00 g, 2.57 mmol) was added at room temperature. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was poured in 200 mL of H_2O,

extracted with AcOEt (3 x 80 mL). The organic layer was washed with brine (2 x 20 mL), dried over $MgSO_4$, filtered and evaporated to dryness. The crude compound was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH = 98:2 to 9:1) to give after recrystallisation in diethyl ether **EHT 6271** (370 mg, 33 % yield) as a white solid.

The structure of compound ex 11 is presented below:



MW: 537.59; Yield: 33 %; White solid; Mp: 67.8 °C.

R_f: 0.20 (EtOAc).

^{1}H-NMR ($CDCl_3$, δ): 1.56-1.90 (m, 14H, 7xCH_2), 3.13 (t, J = 7.3 Hz, 2H, CH_2S), 3.53-3.57 (m, 1H, O-CH_2), 3.79-3.84 (m, 1H, O-CH_2), 3.88 (t, J = 6.4 Hz, 2H, CH_2O), 4.32 (d, J_{BA} = 14.4 Hz, 1H, O-CH_2), 4.52 (d, J_{AB} = 14.4 Hz, 1H, O-CH_2), 4.37 (s, 2H, CH_2O), 4.72 (t, J = 3.0 Hz, 1H, CH), 6.51 (s, 1H, –C=CH), 7.26 (d, J = 4.8 Hz, 1H, Ar-H), 7.56 (s, 1H, –C=CH), 7.71 (dd, J = 8.8 Hz, J = 1.7 Hz, 1H, Ar-H), 8.24 (d, J = 8.7 Hz, 1H, Ar-H); 8.36 (s, 1H, Ar-H), 8.79 (d, J = 4.8 Hz, 1H, Ar-H).

MS-ESI *m/z* (rel. int.): 538.0 ($[MH]^+$, 100).

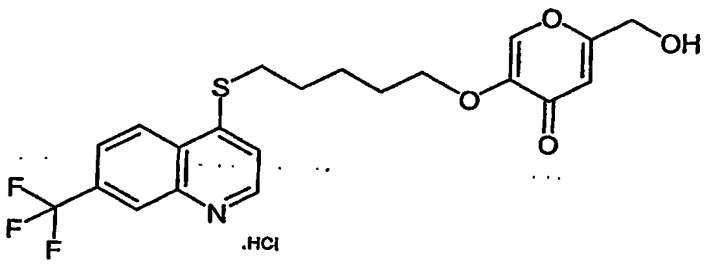
HPLC: Method A, detection UV 254 nm, **EHT 6271**, RT = 5.80 min, peak area 99.8%.

Exemple 12: 2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one hydrochloride salt (EHT 1302).

EHT 9376 (40.6 mg, 0.0077 mmol) was charged in a 5 mL vial equipped with a magnetic stirrer. 2 mL of MeOH and activated DOWEX (50WX8) (50 mg) were added. The reaction mixture was stirred 2 h at room temperature. The

suspension was filtered and the precipitate was washed with a solution of MeOH:HCl 1M = 9:1. After evaporation a viscuous yellow pale oil **EHT 1302** (7 mg, 19 % yield) was obtained.

The structure of compound ex 12 is presented below:



MW: 475.91 (HCl salt); Yield: 19 %; Yellow pale oil.

^{1}H-NMR (CD_3OD, δ): δ 1.60-2.00 (m, 6H, 3xCH_2), 3.42 (t, J = 7.1 Hz, 2H, CH_2S), 3.83 (t, J = 5.8 Hz, 2H, CH_2O), 4.31 (s, 2H, CH_2OH), 6.38 (s, 1H, –C=CH-), 7.92 (s, 2H, –C=CH- and Ar-H), 8.05 (d, J = 8.8 Hz, 1H, Ar-H), 8.33 (s, 1H, Ar-H), 8.57 (d, J = 8.8 Hz, 1H, Ar-H), 8.88 (d, J = 6.0 Hz, 1H, Ar-H).

^{13}C-NMR (CD_3OD): 176.79, 170.72, 166.95, 148.95, 144.63, 144.54, 136.75, 136.68, 136.30, 128.86, 127.64, 126.15, 122.52, 120.24, 120.18, 118.24, 111.70, 70.40, 61.06, 32.84, 29.44, 28.34, 26.42.

MS-ESI *m/z* (rel. int.): 439.95 ([MH]$^+$, 100), 306.08 (10).

HPLC: Method A, detection UV 254 nm, **EHT 1302** RT = 4.63 min, peak area 99.0 %.

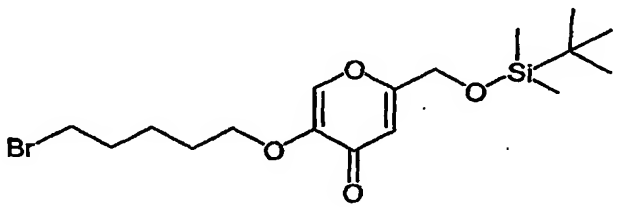
Preparation of EHT 5909, EHT 2168, EHT 1494, EHT 7365 and EHT 7168.

Exemple 13: 5-(5-Bromo-pentyloxy)-2-(*tert*-butyl-dimethyl-silanyloxymethyl)-4*H*-pyran-4-one 4.

2-(*tert*-Butyl-dimethyl-silanyloxymethyl)-5-hydroxy-4*H*-pyran-4-one x (1.50 g, 5.85 mmol) was charged in a 100 mL round-bottomed flask equipped with a magnetic stirrer and under inert atmosphere. Anhydrous DMF (25 mL) and Cs_2CO_3 (2.10 g, 6.44 mmol) were successively added. After 5 min, 1,5-

dibromopentane (2.39 mL, 17.55 mmol) was added via syringe at room temperature. The reaction mixture was heated at 50°C for 3 h. After cooling and filtration DMF was removed *In vacuo*. The crude oil was purified by chromatography on silica using as eluent AcOEt:cyclohexane = 20:80 then 30:70. After evaporation, 5-(5-bromo-pentyloxy)-2-(*tert*-butyl-dimethyl-silanyloxymethyl)-4*H*-pyran-4-one **4** was obtained (1.25 g; 53% yield) as a white solid.

The structure of compound ex 13 is presented below:



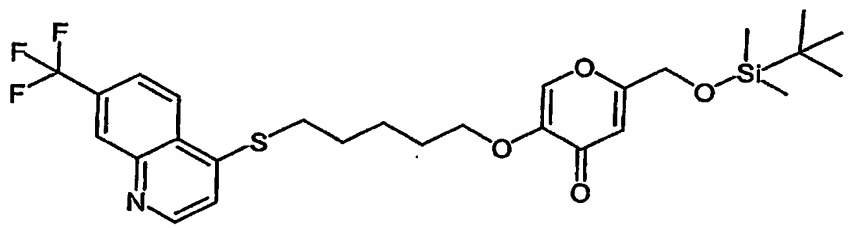
MW: 405.40; Yield: 53 %; White solid; Mp = 65.3 °C.
R_f: 0.65 (AcOEt:Cyclohexane = 50:50).
^{1}H-NMR (CD_3Cl, δ): 0.11 (s, 6H, 2xCH_3), 0.93 (s, 9H, 3xCH_3), 1.52-1.68 (m, 2H, CH_2), 1.79-1.97 (m, 4H, 2xCH_2), 3.43 (t, *J* = 6.7 Hz, 2H, CH_2Br), 3.88 (t, *J* = 6.4 Hz, 2H, CH_2O), 4.46 (s, 2H, CH_2OSi), 6.50 (d, *J* = 0.5 Hz, 1H, –C=CH-), 7.54 (s, 1H, –C=CH).
^{13}C-NMR (CD_3Cl): 174.58, 166.84, 147.81, 139.00, 111.71, 69.38, 61.21, 33.53, 32.36, 28.21, 25.73, 24.58, 18.26, -5.48.

Exemple 14: 2-(*tert*-Butyl-dimethyl-silanyloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one 5.

7-Trifluoromethyl-4-quinoline-thiol (0.77 g, 3.37 mmol) was charged in a 100 mL round-bottomed flask equipped with a magnetic stirrer and under inert atmosphere. Anhydrous DMF (20 mL) and NaH 60% dispersion in oil (135 mg, 3.37 mmol) were successively added. After 40 min a solution of 5-(5-bromo-pentyloxy)-2-(*tert*-butyl-dimethyl-silanyloxymethyl)-4*H*-pyran-4-one **4** (1.24 g,

3.06 mmol) in 10 mL DMF was added *via* syringe at room temperature. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was poured in 500 mL of H_2O, extracted with AcOEt (3 x 150 mL). The organic layer was washed with brine (4 x 250 mL), dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude yellow solid was purified by chromatography on silica using as eluent AcOEt:cyclohexane = 50:50 then 60:40. After evaporation, 2-(*tert*-butyl-dimethyl-silanyloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one **5** was obtained (1.27 g, 75 % yield) as a yellow solid.

The structure of compound ex 14 is presented below:



MW: 553.71; Yield: 75 %; Yellow solid.

R_f: 0.31 (AcOEt:cyclohexane = 60:40).

^{1}H-NMR (CD_3Cl, δ): 0.11 (s, 6H, 2xCH_3), 0.93 (s, 9H, 3xCH_3), 1.60-1.78 (m, 2H, CH_2), 1.80-1.95 (m, 4H, 2xCH_2), 3.15 (t, J = 7.2 Hz, 2H, CH_2S), 3.89 (t, J = 6.2 Hz, 2H, CH_2O), 4.46 (s, 2H, CH_2OSi), 6.51 (d, J = 0.8 Hz, 1H, –C=CH-), 7.26 (d, J = 4.8 Hz, 1H, Ar-H), 7.53 (s, 1H, –C=CH), 7.71 (dd, J = 8.8 Hz, J = 1.4 Hz, 1H, Ar-H), 8.23 (d, J = 8.8 Hz, 1H, Ar-H), 8.36 (s, 1H, Ar-H), 8.79 (d, J = 4.8 Hz, 1H, Ar-H).

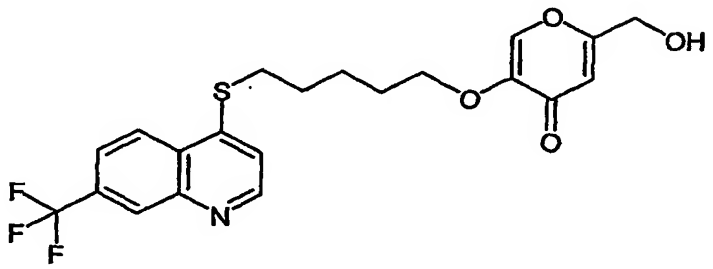
MS-ESI *m/z* (rel. int.): 554.0 ($[MH]^+$, 100).

HPLC: Method A, detection UV 254 nm, RT =6.57.

Exemple 15: 2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 5909).

In a 50 mL round-bottomed flask 2-(*tert*-butyl-dimethyl-silanyloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one **5** (1.20 g, 2.17 mmol) was dissolved in 25 mL of THF. A solution of *n*-tetrabutylammonium fluoride in THF (2.38 mL, 2.38 mmol) was added *via* syringe. The raction mixture was stirred 1 h at RT. The reaction mixture was evaporated *in vacuo* and the crude product was purified by chromatography on silica using as eluent CH_2Cl_2:MeOH = 95:5. After evaporation a yellow light solid **EHT 5909** (0.73 g, 77 % yield) was obtained.

The structure of compound ex 15 is presented below:



MW: 439.11; Yield: 77 %; Yellow pale solid; Mp = 133.2 °C (dec., AcOEt).

R_f: 0.23 (CH_2Cl_2:MeOH = 95:5).

^{1}H-NMR (CD_3Cl, δ): 1.65-1.76 (m, 2H, CH_2), 1.77-1.95 (m, 4H, 2xCH_2), 3.14 (t, *J* = 7.2 Hz, 2H, CH_2S), 3.86 (t, *J* = 8.2 Hz, 2H, CH_2O), 4.12 (s broad, 1H, OH), 4.49 (s, 2H, CH_2OH), 6.52 (s, 1H, –C=CH-), 7.26 (d, *J* = 4.8 Hz ,1H, Ar-H), 7.54 (s, 1H, –C=CH-), 7.70 (dd, *J* = 8.8 Hz, *J* = 1.7 Hz, 1H, Ar-H), 8.22 (d, *J* = 8.8 Hz, 1H, Ar-H), 8.33 (s, 1H, Ar-H), 8.74 (d, *J* = 4.8 Hz, 1H, Ar-H).

^{13}C-NMR (CD_3Cl): 174.70, 167.23, 150.42, 148.56, 147.79, 146.39, 139.29, 128.14, 127.50, 125.00, 121.89, 117.26, 111.98, 69.29, 60.81, 30.99, 28.55, 27.76, 25.40.

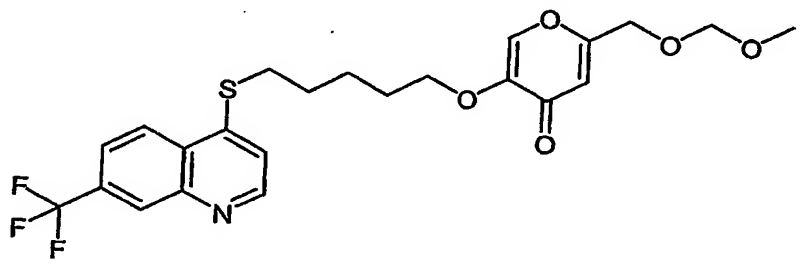
MS-ESI *m/z* (rel. int.): 439.9 ($[MH]^+$, 100), 298.08 (5), 211 (10), 142.9 (5).

HPLC: Method A, detection UV 254 nm, **EHT 5909** RT = 4.60 min, peak area 99.9%.

Exemple 16: 2-Methoxymethoxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 2168).

2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one **EHT 5909** (0.10 g, 0.227 mmol) was charged in a 25 mL three necked round bottom flask equipped with a magnetic stirrer and under a nitrogen atmosphere. THF (6 mL) and NaH (60% dispersion in oil, 10 mg, 0.25 mmol) were added at room temperature. After the reaction mixture was stirred for 5 min, methyl choromethyl ether (18.1 µl, 0.24 mmol, 1.05 eq.) was added *via* syringe and the reaction mixture was stirred for 2 h 30 at room temperature. The raction mixture was poured in H_2O (60 mL) and extracted with AcOEt (3 x 20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude product was purified by chromatography on silica using as eluent MeOH:CH_2Cl_2 = 2:98. After evaporation and drying to the vacuum pump a white pale yellow solid **EHT 2168** was obtained (74 mg, 67 % yield).

The structure of compound ex 16 is presented below:



MW: 483.50; Yield: 67 %; Yellow pale solid; Mp: 88.1 °C.

R_f: 0.42 (CH_2Cl_2:MeOH = 95:5).

^{1}H-NMR (CD_3Cl, δ): 1.73-1.80 (m, 2H, CH_2-CH_2-CH_2), 1.82-1.98 (m, 4H, CH_2-CH_2-CH_2), 3.15 (t, J = 7.2 Hz, 2H, CH_2S), 3.40 (s, 3H, MeO), 3.89 (t, J = 6.2 Hz, 2H, CH_2O), 4.37 (s, 2H, CH_2O), 4.70 (s, 2H, CH_2O), 6.48 (s, 1H, –C=CH-), 7.26 (d, J = 4.8 Hz, 1H, Ar-H), 7.56 (s, 1H, –C=CH-), 7.71 (dd, J = 8.8 Hz, J = 1.7 Hz,

1H, Ar-H), 8.23 (d, J = 8.8 Hz, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 8.79 (d, J = 4.8 Hz, 1H, Ar-H).

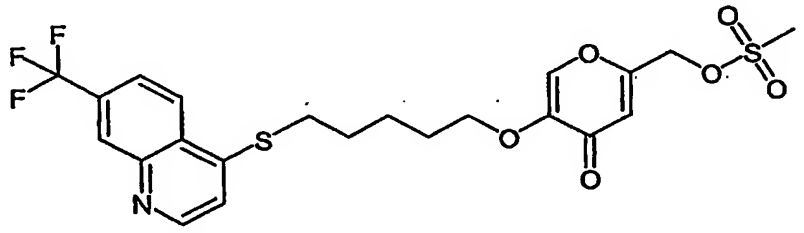
MS-ESI *m/z* (rel. int.): 484.1 ($[MH]^+$, 100), 255 (5).

HPLC: Method A, detection UV 254 nm; **EHT 2168** RT = 5.19 min, peak area 99.9 %.

Exemple 17: Methanesulfonic acid 4-oxo-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-2-ylmethyl ester 6.

To a suspension of 2-hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one **EHT 5909** (0.10 g, 0.23 mmol) in 4 mL of dichloromethane at 5°C were added triethylamine (35 µl, 0.25 mmol) and methanesulfonyl chloride (19.4 µl, 0.25 mmol). The reaction mixture was stirred 18 h at room temperature. Dichloromethane (20 mL) was added and the solution was washed with aqueous $NaHCO_3$ 10% (2x 30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude product was purified by chromatography on silica using as eluent CH_2Cl_2:MeOH = 98:2. After evaporation methanesulfonic acid 4-oxo-5-[6-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-hexyloxy]-4*H*-pyran-2-ylmethyl ester **6** (83 mg, 69 %) was obtained as a white solid.

The structure of compound ex 17 is presented below:



MW: 517.54; Yield: 69 %; White solid; Mp = 122.1 °C (dec.).

R_f: 0.44 (CH_2Cl_2:MeOH = 95:5).

^{1}H-NMR (CD_3Cl, δ): 1.63-1.78 (m, 2H, CH_2), 1.80-1.95 (m, 4H, 2xCH_2), 3.11 (s, 3H, MeS); 3.15 (t, J = 7.2 Hz, 2H, CH_2S), 3.90 (t, J = 6.2 Hz, 2H, CH_2O), 4.98 (s, 2H, CH_2OH), 6.52 (s, 1H, –C=CH-), 7.26 (d, J = 4.8 Hz ,1H, Ar-H), 7.60 (s, 1H, –C=CH-), 7.71 (dd, J = 8.8 Hz, J = 1.8 Hz, 1H, Ar-H), 8.23 (d, J = 8.8 Hz, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 8.79 (d, J = 4.8 Hz, 1H, Ar-H).

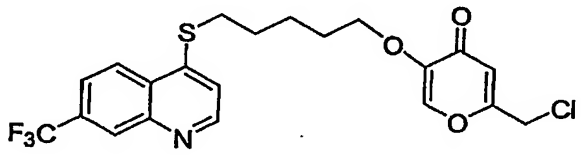
MS-ESI *m/z* (rel. int.): 517.81 ($[MH]^+$, 100).

HPLC: Method A, detection UV 254 nm, RT = 5.04 min, peak area 97.0 %.

Exemple 18: 2-Chloromethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 1494).

The compound was prepared according the above procedure from **EHT 5909** instead that after the reaction, the mixture was evaporated to dryness and let 18 h at room temperature (a spontaneous reaction between the mesylate and triethylamine hydrochloride occurred). Purification by preparative HPLC (CH_3CH:H_2O:TFA 1/1000 gradient) yielded to **EHT 1494** (43%) as a yellow solid.

The structure of compound ex 18 is presented below:



MW: 457.89; Yield: 43 %; Yellow solid; Mp: 38.2 °C.

R_f: 0.35 (CH_2Cl_2:MeOH = 9:1).

^{1}H-NMR ($CDCl_3$, δ): 1.70-2.00 (m, 6H, CH_2), 3.26 (t, J = 6.9 Hz, 2H, CH_2S), 3.92 (t, J = 5.8 Hz, 2H, CH_2O), 4.33 (s, 2H, Cl-CH_2), 6.50 (s, 1H, –C=CH), 7.50 (m, 1H, Ar-H), 7.61 (s, 1H, -C=CH), 7.83 (d, J = 8.8 Hz, 1H, Ar-H), 8.33 (d, J = 8.8 Hz, 1H, Ar-H), 8.59 (s, 1H, Ar-H), 8.92 (m, 1H, Ar-H).

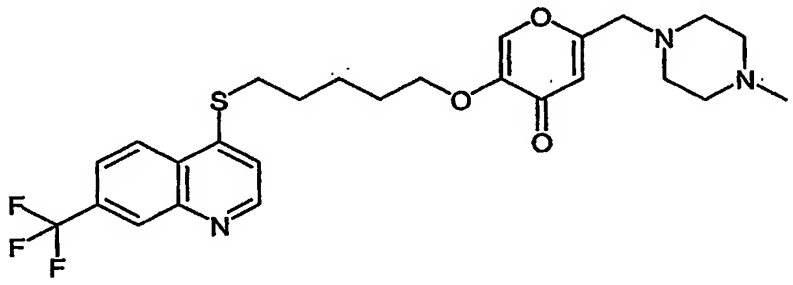
MS-ESI *m/z* (rel. int.): 457.9-459.8 ($[MH]^+$, 100).

HPLC: Method A, Detection UV 254 nm, **EHT 1494**, RT = 5.39 min, purity 94.7%.

Exemple 19: 2-(4-Methyl-piperazin-1-ylmethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7365).

Methanesulfonic acid 4-oxo-5-[6-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-hexyloxy]-4*H*-pyran-2-ylmethyl ester **6** (82 mg, 0.16 mmol) in 1.5 mL of dichloromethane was charged in a 10 mL round-bottomed flask equipped with a magnetic stirrer and under N_2 atmosphere. 1-Methylpiperazine (35.1 µl, 0.32 mmol) was added *via* syringe and the reaction mixture was heated at 45 °C for 2 h 40. The reaction mixture was evaporated to dryness, recrystallized in AcOEt, washed by Et_2O. 40 mL of dichloromethane were added and the solution was washed with successively $NaHCO_3$ at 5% (40 mL) and brine (40 mL). The solution was dried over $MgSO_4$, filtered and evaporated to give a pale yellow solid **EHT 7365** (59 mg, 72 % yield).

The structure of compound ex 19 is presented below:



MW: 521.59; Yield: 72 %; Yellow pale solid; Mp: 112.6 °C (dec.).

R_f: 0.26 (CH_2Cl_2:MeOH = 90:10).

^{1}H-NMR (CD_3Cl, δ): 1.61-1.79 (m, 2H, CH_2-CH_2-CH_2), 1.82-1.98 (m, 4H, CH_2-CH_2-CH_2-CH_2), 2.31 (s, 3H, CH_3N), 2.20-2.75 (m, 8H, CH_2-N-CH_2 and CH_2-N(CH_3)-CH_2), 3.15 (t, J = 7.2 Hz, 2H, CH_2S), 3.37 (s, 2H, =CCH_2N), 3.89 (t, J = 6.2 Hz, 2H, CH_2O), 6.45 (s, 1H, –C=CH-), 7.26 (d, J = 4.8 Hz ,1H, Ar-H), 7.56 (s, 1H, –

C=CH-); 7.71 (dd, J = 8.8 Hz J = 1.7 Hz, 1H, Ar-H), 8.23 (d, J = 8.8 Hz, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 8.79 (d, J = 4.8 Hz, 1H, Ar-H).

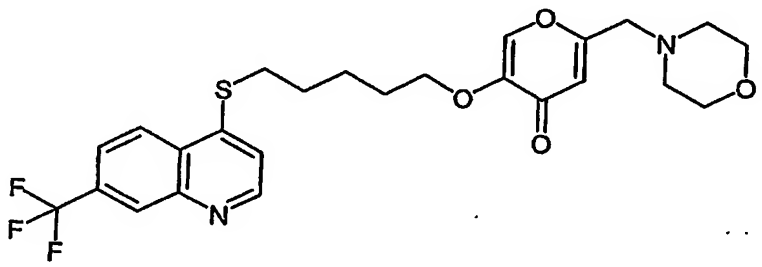
MS-ESI *m/z* (rel. int.): 521.9 ($[MH]^+$, 100); 297.9 (20), 282.1 (20), 229.9 (30).

HPLC: Method A, detection UV 254 nm, **EHT 7365** RT = 4.12 min, peak area 98.4 %, impurity RT= 5.24, 1.6 %.

Exemple 20: 2-Morpholin-4-ylmethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7168).

Methanesulfonic acid 4-oxo-5-[6-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-hexyloxy]-4*H*-pyran-2-ylmethyl ester **6** (96 mg, 0.184 mmol) in 1.8 mL of dichloromethane was charged in a 10 mL round-bottomed flask equipped with a magnetic stirrer and under N_2 atmosphere. Morpholine (41 µl, 0.46 mmol) was added *via* syringe and the reaction mixture was heated at 45 °C for 3 h 30. The reaction mixture was evaporated to dryness, recrystallized in AcOEt, washed by Et_2O. 40 mL of dichloromethane were added and the solution was washed with successively $NaHCO_3$ at 5% (40 mL) and brine (40 mL). The solution was dried over $MgSO_4$, filtered and evaporated to give a pale yellow solid **EHT 7365** (60 mg, 64 % yield).

The structure of compound ex 20 is presented below:



MW: 508.55; Yield: 64%; Yellow light solid; Mp= 93.3 °C.

R_f: 0.33 (AcOEt)

^{1}H-NMR ($CDCl_3$, δ): 1.72-1.74 (m, 2H, CH_2), 1.88-1.92 (m, 4H, 2xCH_2), 2.51-2.52 (m, 4H, CH_2-N-CH_2), 3.13-3.18 (t, J = 7.0 Hz, 2H, -SCH_2), 3.36 (s, 2H, -NCH_2), 3.72-3.73 (m, 4H, CH_2-O-CH_2), 3.87-3.91 (t, J = 6.0 Hz, 2H, O-CH_2), 6.47 (s, 1H,

-CH=C), 7.26 (m, 1H, Ar-H), 7.56 (s, 1H, -CH=C), 7.69-7.72 (d, J = 8.8 Hz, 1H, Ar-H), 8.23-8.25 (d, J = 8.7 Hz, 1H, Ar-H), 8.36 (s, 1H, Ar-H), 8.78-8.80 (d, J = 4.7 Hz, 1H, Ar-H).

MS-ESI *m/z* (rel. Int.): 509.1 ($[MH]^+$, 100).

HPLC: Method A, detection UV 254 nm, **EHT 7168** RT = 4.37 min, peak area 99.9 %.

Example 21 : Pharmacology

This example discloses the various assay conditions used to illustrate the biological activity of the compounds.

Material and Methods

A series of *in vitro* tests was designed to screen the various compounds and evaluate their anti-proliferative, anti-tumoral and anti-angiogenic potential. L651582 (carboxyamidotriazole; CAI, Merck) and Paclitaxel (taxol, Bristol Myers Squibb) were included as positive controls.

Reference L651582 (Merck Institute for Therapeutic Research, Rahway, NJ) is a carboxyamide-amino-imidazole compound originally developed as a coccidiostat (U.S. patent No. 4,590,201). L651582 has been shown later by the NCI to be a synthetic inhibitor of both nonvoltage- and voltage-gated calcium pathways. It demonstrated inhibition of tumor cell motility, adhesion, metastatic potential, and growth *in vitro* in a number of human tumor cell lines at concentrations from 1 to 10 µM. L651582 was also demonstrated to inhibit proliferation, migration and adhesion of several human endothelial cells. Moreover, inhibition of both HUVEC vascular tube formation on matrigel and retardation of microvessel outgrowth in the rat aorta ring were shown. Finally, *in vivo* inhibition of angiogenesis in the chicken chorioallantoic membrane assay at concentrations from 10 to 20 µM was also demonstrated.

Cell culture and cell viability assay

In order to determine one compound effect on cell viability, microculture tetrazolium assay (MTT) was performed as described by Carmichael *et al.* (1996)

with modifications. Four human tumoral cell lines, namely HCT116 colon adenocarcinoma, H460 lung carcinoma, MCF-7 and MDA-MB-231 breast carcinoma cell lines, and 3 immortalized but non tumorigenic cell lines, namely NIH3T3 mouse fibroblasts, human breast-derived MCF10-A, human lung-derived MRC-5 were purchased at the American Type Culture Collection (ATCC), Manassas, USA and cultured according to their recommendations. Human Microvasculature Endothelial Cell line HMEC1 was obtained from the National Center for Infectious Diseases, Atlanta, USA. Briefly, 2.5 10^3 to 2 10^4 cells per well were seeded in 48-well plates 24 hours before drug addition. Cells were treated with 0 to 200 µM (11 concentrations) of compound solubilized in DMSO, adjusting the final concentration of DMSO to 1% in the well. Six days after treatment, the medium was replaced by PBS containing 0.5 mg/ml MTT (Sigma) and cells were incubated for 1-3 hours at 37°C before solubilization of formazan crystals in 100% DMSO. Absorbance was measured using a spectrophotometer at a wavelength of 550nm. Data was analyzed using the GraphPad Prism software (GraphPad Software, Inc., San Diego, USA), and IC50 (dose leading to 50% cell death) was calculated from the dose-response curves.

Anchorage-independent cell growth assay in soft agar

In order to evaluate the effect of one compound on the capacity of tumour cells to grow without anchorage, HCT116 cells were seeded in soft agar. In contrast to microplate assays which average the drug's effects over an entire cell population, clonogenic assays offer the possibility of distinguishing cytotoxic agents (i.e., decreased colony number) from cytostatic agents (i.e., decreased colony size without decreased colony number ; Murphy M.J. *et al.*, 1996).

Briefly, 5 10^3 HCT116 cells were resuspended in 300 µl of complete medium containing 0.3% soft-agar (Difco) and different concentrations of compound (8 concentrations ranging from 0 to 30 µM). Cells were then poured on a solidified layer of medium containing 0.5% of soft agar plus the compound at the same concentration as in the upper layer. Cells were incubated for 7 days at 37°C before pictures of each well were taken using a phase contrast microscope (Nikon) and a digital camera (Nikon Coolpix 990). Pictures were subsequently

analyzed using a free image analysis software from the NIH (ImageJ) allowing to determine clones size and number.

Data was analyzed using the GraphPad Prism software, and IC50 (dose leading to a 50% decrease of clone size or number) was calculated from dose-response curves. The ratio IC50 clone size/IC50 clone number was then calculated. When this ratio is equal to 1 (IC50 size=IC50 number), the compound is referred to as "cytotoxic". When the ratio is close to 0 (IC50 size<<IC50 number), the compound is referred to as "cytostatic" (Figure 1).

Migration

An essential characteristic of malignant cells is their ability to migrate, invade host tissues and to produce metastases. In order to evaluate the capacity of one compound to affect the ability of tumoral cells to migrate, migration assays were performed using highly invasive tumoral cells MDA-MB-231. This assay was performed using Falcon HTS Fluoroblock inserts. Culture medium containing Fetal Bovine Serum (FBS; which is used as a chemoattractant) was added to the plate wells and 2 10^4 MDA-MB-231 cells resuspended in medium without FBS and with 0.1% BSA were added to each insert well. The compound of interest was added to the medium in both the upper and the lower chambers. Plates were incubated for 16 hours at 37°C. Following incubation, the medium was removed from the upper chamber and the entire insert plate was transferred to a second 24-well plate containing 4 µg/ml Calcein-AM in medium containing 0.1% BSA. The plates were incubated for one hour at 37°C, rinsed with Hanks Buffered Saline (HBSS). Fluorescence data were collected using Fluoroskan Ascent FL fluorescence plate reader at an excitation wavelength (Ex) of 485 nm and emission wavelength (Em) of 517 nm. Only those labelled cells that passed through the Matrigel layer and the membrane were detected. Data were analyzed using the GraphPad Prism software.

Tubule formation assay

The endothelial cell tube formation assay, or tubulogenesis assay, is an *in vitro* assay that is widely accepted to accurately reflect number of terminal stages of the angiogenic process such as attachment, migration and differentiation into

tubular structures. In this model, endothelial cells are cultured on a monolayer of reconstituted basement membrane components (Matrigel) and form, in a few hours, capillary-like structure. Therefore, this system is a useful and powerful tool for rapidly screening anti-angiogenic agents by monitoring inhibition of endothelial cells morphogenesis on extracellular matrix.

HMEC-1 cell line is cultured in MCDB-131 medium (Sigma) supplemented with 15% FBS, 10 ng/ml recombinant human epidermal growth factor (Invitrogen) and 1 µg/ml hydrocortisone. Cells are cultured to 70-80% confluence. A 96-well plate was coated with Matrigel® Basement Membrane Matrix (Becton Dickinson). 2 10^4 HMEC-1 cells were seeded into each well. The test of control compounds were diluted in DMSO and added to the coated plate. The plate was subsequently incubated for 6 to 8 hours at 37°C, 5% CO_2 atmosphere to allow tubule formation. The cells were then incubated with 1.25 µg/ml Calcein AM for 15 minutes at 37°C, 5% CO_2. Pictures of each well were taken using a fluorescent microscope and a digital camera (Nikon Coolpix 990). Images were analysed using AngioSys software (TCS Cellworks, Buckingham, UK) that allows quantification of the *total tubule length* and of the *number of junctions,* two parameters representative of tubule formation/disruption. Data was analyzed using the GraphPad Prism software, and IC50s was calculated. Results were expressed as the ratio "IC50 L651582/IC50 compound".

Results

1. MTT assay in tumoral and endothelial cell lines

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In order to determine the capacity of the compounds to affect cell viability, MTT assays using a tumoral cell line (HCT116) and an endothelial cell line (HMEC1) were performed as described in the material and methods. IC50s were calculated and the results are shown on Figure 2.

Compounds with various substitutions on the aromatic moiety (EHT 9376, EHT 9014, EHT 5810): In HCT116 cell line, it was shown that best compound is

EHT 9376 (IC50 = 0.8±0.2 µM), followed by EHT 1593 (IC50 = 2.4+0.1 µM), EHT 9014 (IC50 = 5.9 µM), and EHT 5810 (IC50 = 7.7 µM).

Compounds with variations around the linker moiety: Three compounds were tested: EHT 4745 (4 carbons), EHT 9376 (5 carbons) and EHT 6271 (6 carbons). In HCT116, the optimal linker structure is the 5 carbon linker (IC50 = 0.8±0.2 µM), followed by the 6 carbon linker (IC50 = 1 µM). The compound showing the lowest activity is the 4 carbon linker compound (IC50 = 3.8±0.2 µM). In HMEC1, it was also shown that increasing or decreasing the linker length of one carbon does not help increase the efficiency of compounds in affecting the cells viability. EHT 4745 and EHT 6271 have an IC50 of 9 µM against 7 µM for EHT 9376.

Compounds with a 5 carbon linker and bearing a 7-trifluoromethyl-quinolin-4-ylsulfanyl moiety with various substitutions at position 6 of the kojic acid moiety: In HCT116, the compound showing the highest effect on cell viability is EHT 9376 (O-THP group ; IC50 = 0.8±0.2 µM), followed by EHT 7168 (morpholine group; IC50 = 2 µM), EHT 7365 (*N*-methylpiperazine group; IC50 = 3.6±0.4 µM) and finally EHT 2168 (MOM; IC50 = 4.4±0.4 µM). In HMEC1 cells, EHT 9376 (IC50 = 7±1 µM) has a higher activity as compared to EHT 7365 (IC50 = 8 µM) and EHT 7168 (IC50 = 20 µM).

Comparison between EHT 9376 (X = S) and EHT 3788 (X = O): In HCT116, EHT 9376 is 3-fold more effective at decreasing the cells viability (IC50 = 0.8±0.2 µM and 2.3 µM, respectively). In HMEC1 cells, EHT 9376 and EHT 3788 have very similar activities (IC50s of 7 µM).

Interestingly, the compounds of the present invention had an activity 2 to 9-fold higher in HCT116 cells as compared to HMEC1 cells, showing that compounds are more toxic for tumoral cells (rapidly dividing cells) than for endothelial cells (slowly dividing cells).

2. Anchorage-independent growth assay

In order to study the compounds' effects on the ability of HCT116 cells to grow independently from anchorage, cells were grown in soft agar in the presence of various concentrations of the compounds. These experiments allowed 1) to rank the compounds according to their potential in affecting the clone size and 2) to evaluate their mode of action (cytotoxic vs cytostatic).

EHT 9376, EHT 9014, EHT 3788 and EHT 1593 all affect the ability of HCT116 to grow independently from anchorage in the micromolar range. The compound showing the highest effect was EHT 9376. The compound showing the lowest effect was EHT 1593. IC50 for EHT 9376 is very similar to IC50 calculated for reference compound L651582 (5.4±1.2 µM against 5.8±1.0 µM respectively) (Figure 3).

In our experiments, L651582 was shown to preferentially affect clone size as compared to clone number (Figure 4). This is in accordance with the literature where L651582 is described as a cytostatic compound (Wasilenko et al, 1996). Similarly, the best compounds of the invention that were tested preferentially affect clone size. In conclusion, the compounds described in this invention have a cytostatic mode of action.

3. Anti-proliferative effect of EHT 9376 and its reversibility on HCT116 cells measured by MTT

The above data suggest that EHT 9376 causes cytostatic growth suppression. To examine this possibility, the ability of HCT116 cells to resume growth after compound withdrawal from growth inhibited cell cultures was evaluated (Fig. 5). Proliferation of HCT116 cells began following the removal of EHT 9376 and L651582 from the cell cultures after day 3, in a very similar way. These experiments allowed to confirme that EHT 9376 has a mode of action very

similar to the mode of action of L651582, which is described as cytostatic in the literature.

4. Migration assay in tumoral cell line MDA-MB-231

Compound EHT 9376 was tested through migration assay, in parallel with reference compound L651582, which is described in the literature as an anti-migratory compound (Kohn EC et al, 1990; Rust WL et al, 2000). The results are presented in Figure 6.

In our system, 10 µM L651582 was shown to decrease the migration of MDA-MB-231 cells of about 40%. Complete inhibition was obtained with 50 µM of compound. EHT 9376 was shown to similarly affect MDA-MB-231 cells migration: 10 µM EHT 9376 inhibits the cell migration of about 60%, and 50 µM almost completely inhibits cell migration. EHT 9376 is shown here to be an efficient anti-migratory compound.

5. Tubulogenesis assay

As seen above, the compounds of the present invention and L651582 have very close anticancer characteristics (cell proliferation, migration, anchorage independent cell growth). Interestingly, L651582 is described in the literature as being both an anticancer and an anti-angiogenic compound (Bauer KS et al, 2000; Krüger EA & Figg WD, 2001). In order to see if our compounds also share anti-angiogenic characteristics with L651582, a tubulogenesis assay on HMEC1 cells was performed using EHT 4745, EHT 6271, EHT 7365, EHT 7168, EHT 1593, EHT 9376 and EHT 3788.

When plated on Matrigel, HMEC1 cells form vessel-like structures in the presence of medium plus solvent DMSO alone. In contrast, the addition of compounds in culture medium caused a dose-dependent inhibition of Matrigel-induced network formation. IC50 for both *total tubule length* and *number of*

junctions (two parameters which are representative of the tubulogenesis process) were calculated for all the tested compounds using GraphPad Prism, and these IC50s were normalized against the IC50 of L651582 (Figure 7).

It was shown that EHT 3788 is the compound inhibiting the formation of tubule in the greater extent (IC50s being 6 and 7-fold lower as compared to IC50s calculated for L651582). All the other compounds were shown to have an equivalent effect on tubulogenesis (IC50s being 3 to 5-fold Lower for our compounds as compared to L651582).

The inhibition of the tubule formation cannot be attributed to compound toxicity since the endothelial cells are treated for only a few hours and the compounds were shown to have only a moderate effect on HMEC1 cells viability after a 6-day treatment (see MTT results).

These results thus illustrate the ability of the compounds of this invention (and the particular efficacy of compounds) especially of EHT 9376 to affect tumor cell viability, inhibit growth of tumor cells, to affect specific characteristics of tumor cells such as anchorage-independent growth, migration and, especially EHT 3788, to inhibit the formation of tubule (vessel-like structures).

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Anal Biochem. 2000 Apr 10;280(1):11-9.

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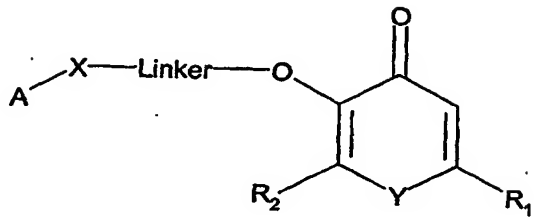
CLAIMS

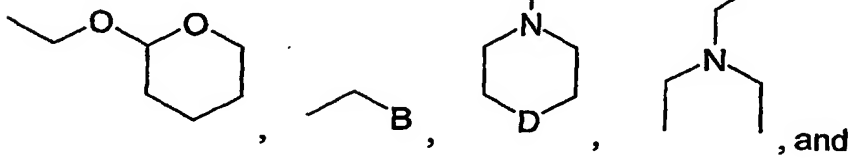
1- A compound having a general formula (I) :



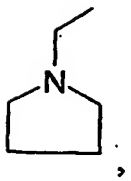
(I)

wherein:

R_1 is selected from the group consisting of :



, and



,

R_2 represents a hydrogen atom, an alkyl or alkenyl group containing from 3 to 6 carbon atoms;

B represents an halogen atom, preferably chlorine, a hydroxyl group, a -O-CH_2-O-CH_3 (MOM) group, a -O-CH_2-O-CH_2-CH_2-O-CH_3 (MEM) group, a -OSO_2-alkyl group or a –$OSi(CH_3)_2tBu$;

D represents an oxygen atom, NR_3, CR'R" or a sulfur atom;

X represents an oxygen atom, a sulfur atom or a radical –NR_4-;

Y represents an oxygen atom, a sulfur atom or a radical –NR_4- ;

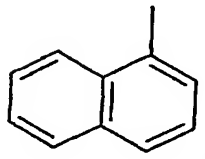
R_3 represents a hydrogen, an alkyl group or a SO_2-alkyl group;

R' and R", identical or different, represent an hydrogen atom or an alkyl radical;

R_4, identical or different, is selected from a group consisting of a hydrogen atom, an alkyl group having from 1 to 10 carbon atoms, an aryl and an aralkyl ;

"linker" represents $(CH_2)_n$, wherein n represents an integer between 1 and 10 inclusive or a aryldialkyl (preferably xylenyl) group;

A represents a group selected from :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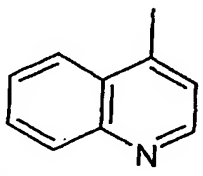
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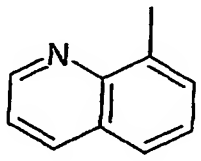
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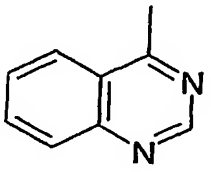
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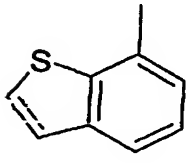


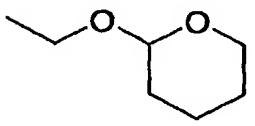
and ,

optionnally A is substituted,

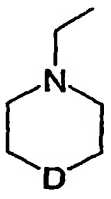
its tautomers, optical and geometrical isomers, racemates, salts, hydrates and mixtures thereof.

2- A compound according to claim 1, wherein :

- X is sulfur or preferably oxygen ; and/or
- Y is oxygen ; and/or
- "linker" represents $(CH_2)_n$, wherein n is from 4 to 7 inclusive, or the xylenyl group ; and/or
- R_1 is



, or



, wherein D is oxygen, $-CH_2-$ or NR_3, wherein R_3 preferably represents an alkyl group (said alkyl is more specifically a methyl radical), and-CH_2-B, wherein B is a $-O-CH_2-O-CH_3$ group or $-OSO_2$-alkyl group (wherein alkyl is preferably methyl) or halogen (preferably chlorine); and/or

- R_2 is a hydrogen atom; and/or
- A is a substituted group.

3- A compound according to claim 1 or 2, wherein A is substituted with at least one substituent, which may be selected from the group consisting in : a hydrogen atom, a halogen atom (preferably F, Cl, or Br), a hydroxyl group, a (C_1-C_{10})alkyl group, an alkenyl group, an (C_1-C_{10})alkanoyl group, a (C_1-C_{10})alkoxy group, a (C_1-C_{10})alkoxycarbonyl group, an aryl group, an aralkyl group, an arylcarbonyl group, a mono- or poly-cyclic hydrocarbon group, a $-NHCO(C_1-C_6)$alkyl group, $-NO_2$, -CN, a $-NR_5R_6$ group or a trifluoro(C_1-C_6)alkyl group, R_5 and R_6, independently from each other, are selected from the group consisting of a

hydrogen atom, an alkyl group having from 1 to 10 carbon atoms, an aryl and an aralkyl.

4. A compound according to claim 3, wherein A is a substituted group and at least one of the substituents is an halogen atom, more preferably chlorine or fluorine.

5- A compound according to claim 4, wherein at least two substituents simultaneously represent Cl, in particular when A is a naphtalene group.

6- A compound according to one of the preceding claims, wherein at least one of the substituents, and more preferably all the substituents, of A represents a hydrogen atom, a methyl group, a propyl group, an ethoxy group, an halogen atom, preferably chlorine or fluorine, or the CF_3 group.

7- A compound according to claim 1, which is selected from the group consisting of :

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-pyran-4-one (EHT 3788)

5-[5-(6-Fluoro-2-methyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1593)

5-[5-(6-Fluoro-2-trifluoromethyl-quinolin-4-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 1074)

5-[5-(7-Propyl-quinolin-8-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 5810)

6,8-Dichloro-4-{5-[4-oxo-6-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-3-yloxy]-pentyloxy}-naphthalene-2-carboxylic acid ethyl ester (EHT 0470)

5-[5-(Benzo[*b*]thiophen-7-yloxy)-pentyloxy]-2-(tetrahydro-pyran-2-yloxymethyl)-4*H*-pyran-4-one (EHT 6060)

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 9376)

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[4-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-butoxy]-4*H*-pyran-4-one (EHT 4745)

2-(Tetrahydro-pyran-2-yloxymethyl)-5-[6-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-hexyloxy]-4*H*-pyran-4-one (EHT 6271)

2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one hydrochloride salt (EHT 1302)

2-Hydroxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 5909)

2-Methoxymethoxymethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 2168)

2-Chloromethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 1494)

2-(4-Methyl-piperazin-1-ylmethyl)-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7365)

2-Morpholin-4-ylmethyl-5-[5-(7-trifluoromethyl-quinolin-4-ylsulfanyl)-pentyloxy]-4*H*-pyran-4-one (EHT 7168).

8- A compound according to claim 7, which is EHT9376 or EHT3788.

9- A pharmaceutical composition comprising at least one compound according to any one of the preceding claims and a pharmaceutically acceptable vehicle or support.

10- A composition according to the preceding claim, for the treatment of a disease associated with abnormal cell proliferation or of a disease associated with unregulated angiogenesis.

11. A composition according to the preceding claim, for the treatment of cancers, restenosis, arthritis, diabetes, ocular-diseases or especially retinopathies.

12- A composition according to one of the preceding claims, for the treatment of solid tumors or lymphoid tumors.

13- A composition according to claim 11, wherein the cancer is selected from prostate cancer, ovarian cancer, pancreas cancer, lung cancer, breast cancer, liver cancer, head and neck cancer, colon cancer, bladder cancer, non-Hodgkin 's lymphoma cancer and melanoma.

14- A composition according to claim 9, for reducing cancer cell proliferation.

15- A composition according to claim 9, for treating metastatic cancers in a subject.

16- A composition according to claim 11, for treating diabetic retinopathies, retinal degenerative diseases, or Age-Related Macular Degeneration (ARMD).

Novel compounds and methods of treating cell proliferative diseases, retinopathies and arthritis

EXONHIT THERAPEUTICS SA

ABSTRACT

The present invention relates to compounds and their uses, particularly in the pharmaceutical industry. The invention discloses compounds having anti-proliferative and antiangiogenic activities, as well as methods for treating various diseases associated with abnormal cell proliferation, including cancer, or associated with unregulated angiogenesis including growth and metastasis of solid tumors, ocular diseases and especially retinopathies, or arthritis, by administering said compounds. It further deals with pharmaceutical compositions comprising said compounds, more particularly useful to treat cancers, ocular diseases and arthritis.

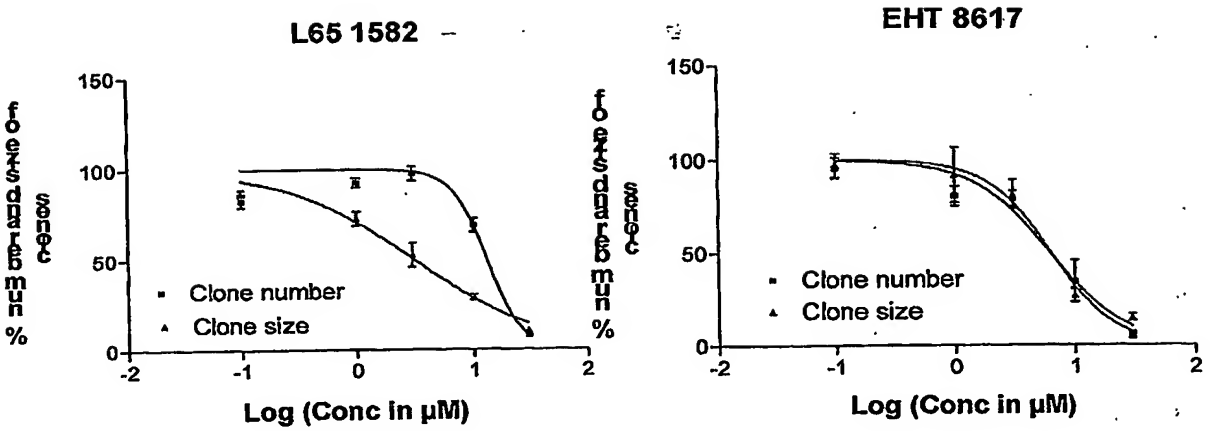
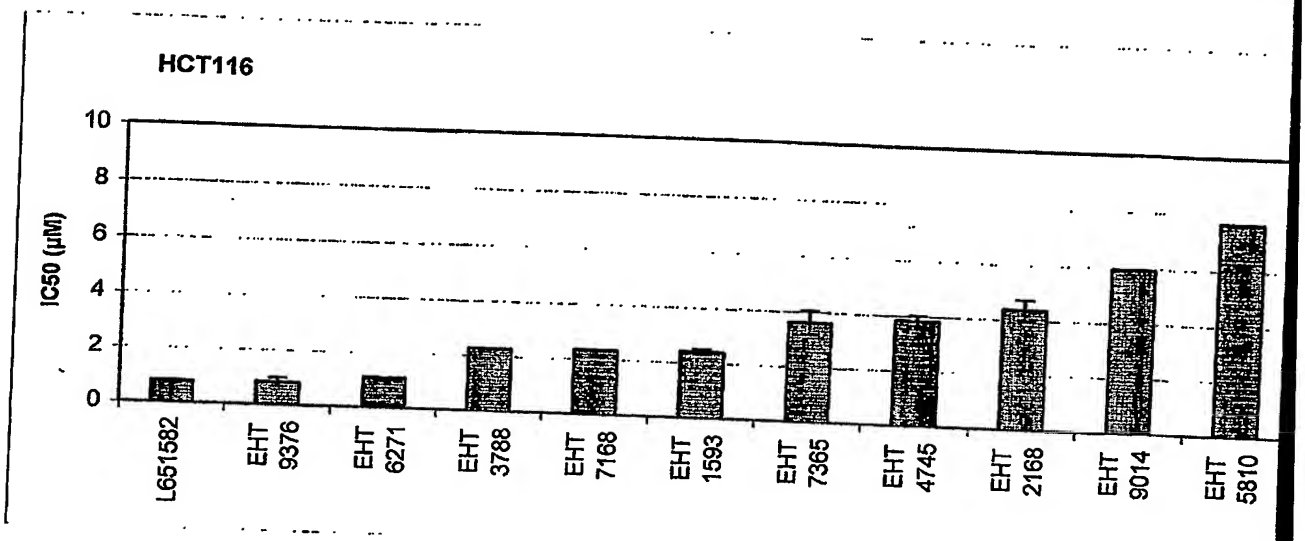

L65 1582
EHT 8617
150
100
50
0
-2
-1
0
1
2
Clone number
Clone size
Log (Conc in µM)

FIGURE 1


HCT116
IC50 (µM)
10
8
6
4
2
0
L651582
EHT 9376
EHT 6271
EHT 3788
EHT 7168
EHT 1593
EHT 7365
EHT 4745
EHT 2168
EHT 9014
EHT 5810

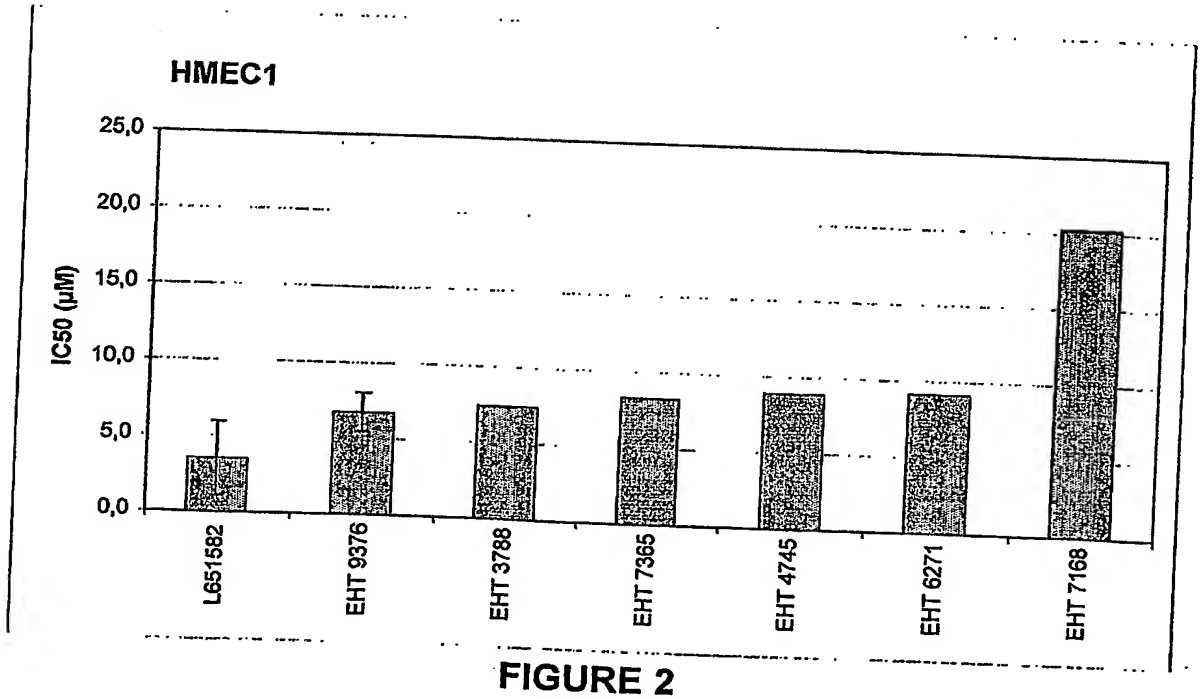

HMEC1
IC50 (µM)
25,0
20,0
15,0
10,0
5,0
0,0
L651582
EHT 9376
EHT 3788
EHT 7365
EHT 4745
EHT 6271
EHT 7168

FIGURE 2

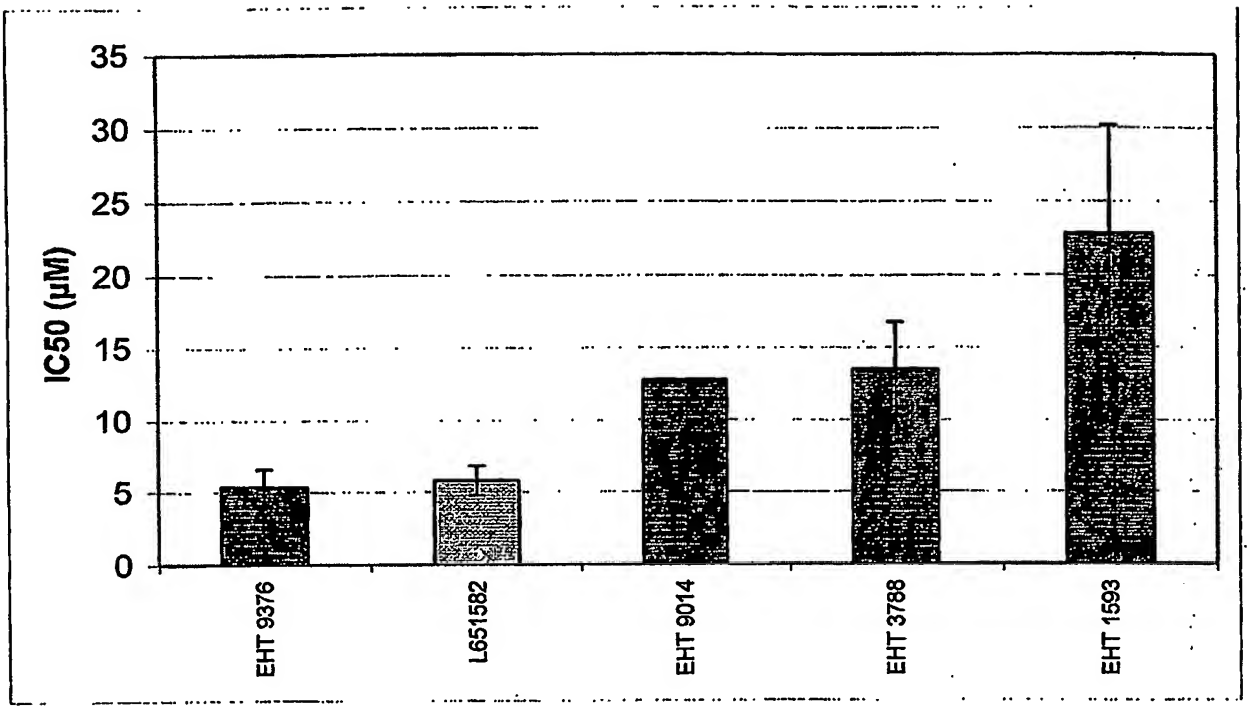

35
30
25
20
15
10
5
0
IC50 (µM)
EHT 9376
L651582
EHT 9014
EHT 3788
EHT 1593

FIGURE 3

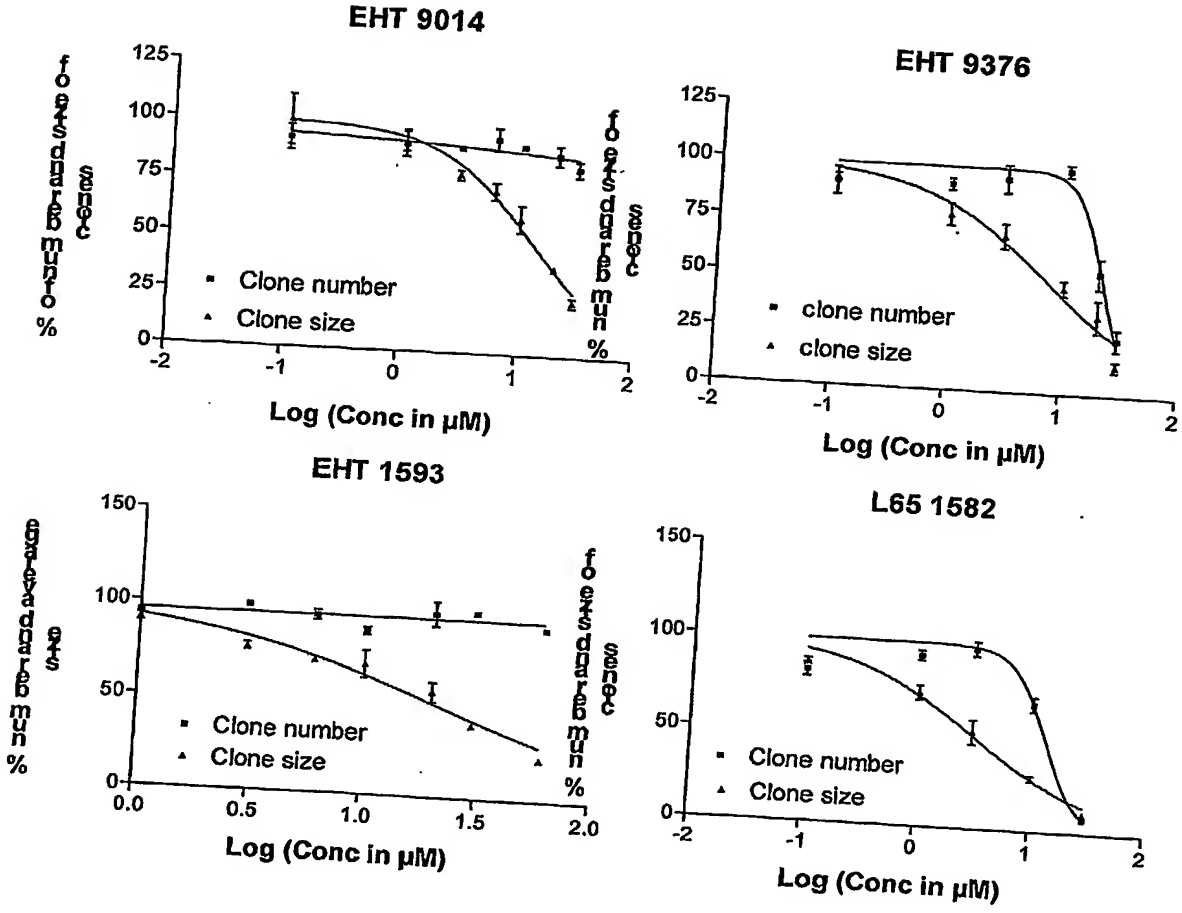

EHT 9014
% of standard response
125
100
75
50
25
0
-2
-1
0
1
2
Clone number
Clone size
Log (Conc in µM)
EHT 9376
% of standard response
clone number
clone size
Log (Conc in µM)
EHT 1593
% of standard response
150
100
50
0
0.0
0.5
1.0
1.5
2.0
Clone number
Clone size
Log (Conc in µM)
L65 1582
% of standard response
Clone number
Clone size
Log (Conc in µM)

FIGURE 4

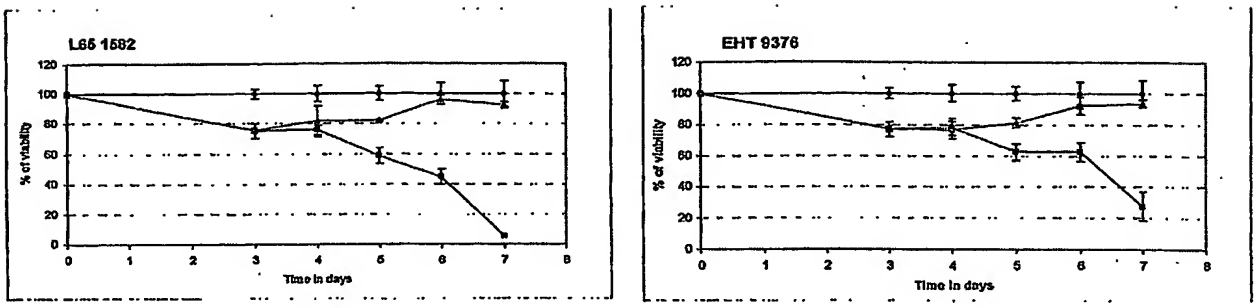

L65 1582
% of viability
Time in days
EHT 9376
% of viability
Time in days

FIGURE 5

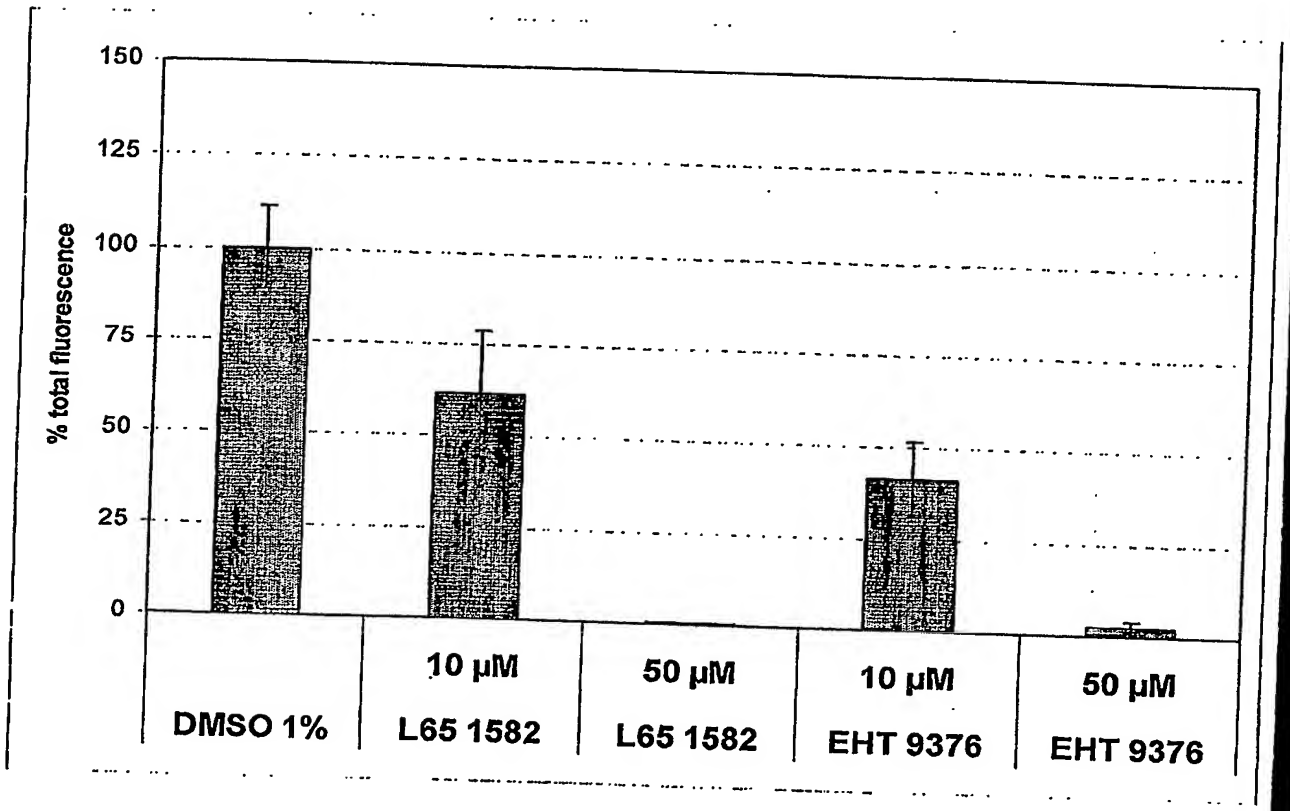

150
125
100
75
50
25
0
% total fluorescence
DMSO 1%
10 µM L65 1582
50 µM L65 1582
10 µM EHT 9376
50 µM EHT 9376

FIGURE 6

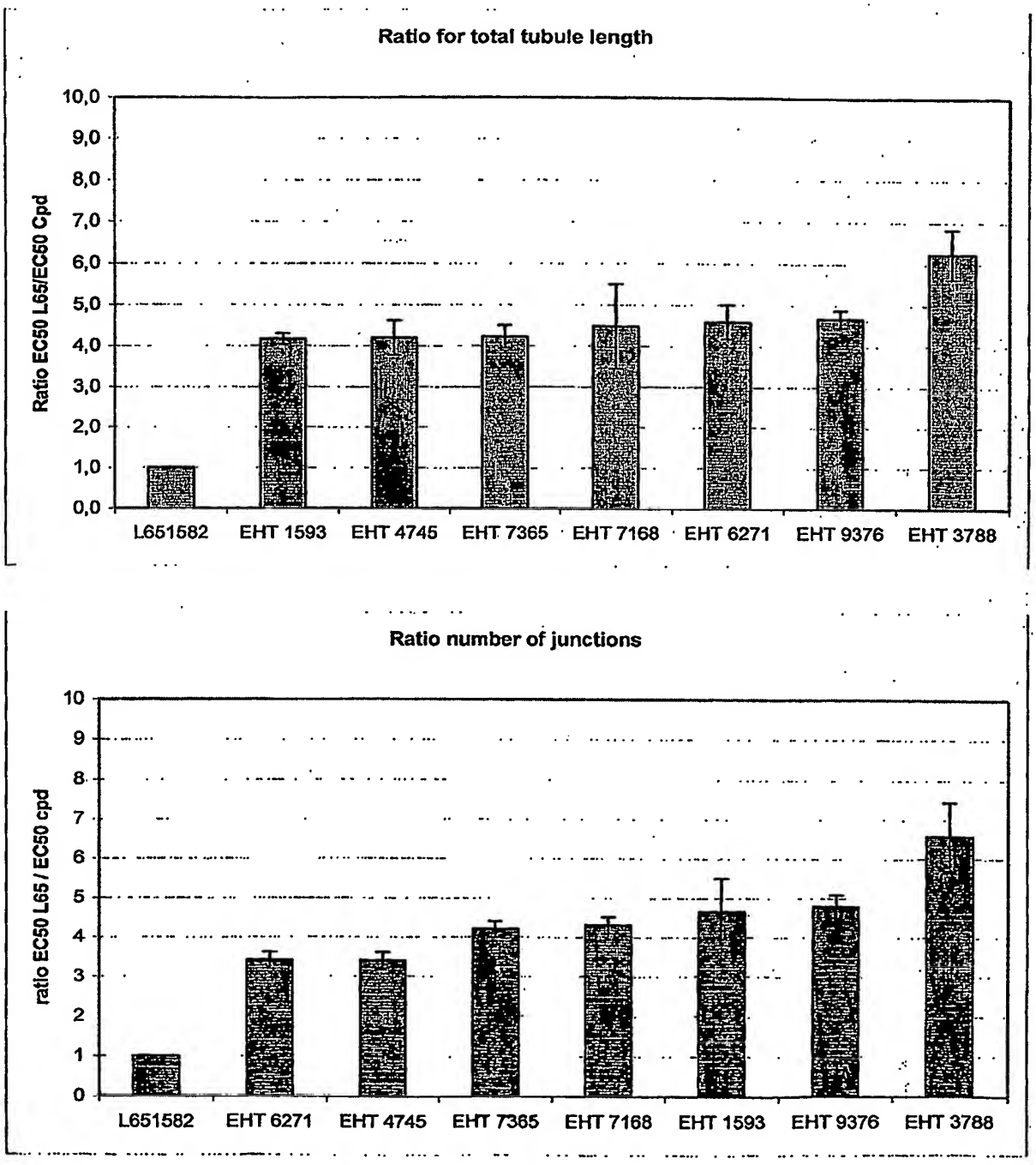

Ratio for total tubule length
Ratio EC50 L65/EC50 Cpd
10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
L651582
EHT 1593
EHT 4745
EHT 7365
EHT 7168
EHT 6271
EHT 9376
EHT 3788
Ratio number of junctions
ratio EC50 L65 / EC50 cpd
10
9
8
7
6
5
4
3
2
1
0
L651582
EHT 6271
EHT 4745
EHT 7365
EHT 7168
EHT 1593
EHT 9376
EHT 3788

FIGURE 7